ผลลดไข้ของสิ่งสกัดจากรากทั้งห้าชนิดของตำรับยาเบญจมูลใหญ่ในหนูแรท

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ANTIPYRETIC EFFECT OF FIVE ROOT EXTRACTS OF BEN-CHA-MOON-YAI REMEDY IN RATS

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A Thesis Submitted in Partial Fulfillment of the Requirements
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ตำรับยาเบญจมูลใหญ่เป็นตำรับยาแพทย์แผนไทยมีสรรพคุณในการลดไข้ แก้อักเสบ ซึ่งประกอบไปด้วยรากของสมุนไพรคือ รากมะตูม รากเพกา รากแคแตร รากลำไย และรากคัดลิ้น การทดลองครั้งนี้เป็นการทดสอบฤทธิ์ลดไข้ของสิ่งสกัดจากรากของตำรับยาเบญจมูลใหญ่ (BMY) *Aegle marmelos* (AM) *Oroxylum indicum* (OI), *Dolichandrone serrulata* (DS), *Dimocarpus longan* (DL) และ *Walsura trichostemon* (WT) ด้วยวิธีการเหนี่ยวนำให้หนูแรทเป็นไข้ด้วยไลโพโพลีแซคคาไรด์ (LPS) โดยสัตว์ทดลองจะถูกเหนี่ยวนำให้เป็นไข้ด้วยการฉีด LPS ขนาด 50 ไมโครกรัม/กก. เข้าทางกล้ามเนื้อ หลังจากป้อน 2% Tween 80, แอสไพริน ขนาด 300 มก./กก. หรือ BMY ขนาด 125, 250 และ 500 มก./กก และ AM, OI, DS, DL, WT ขนาด 25, 50, 100, 200 และ 400 มก./กก. ไปแล้ว 1 ชม. โดยจะวัดอุณหภูมิของหนูทางทวารหนักก่อนให้สารและหลังฉีด LPS ทุกชั่วโมงเป็นเวลา 7 ชั่วโมง พบว่า BMY ทุกขนาดที่ทำการทดสอบ AM และ OI ขนาด 400 มก./กก. และ WT 100 มก./กก. สามารถลดอุณหภูมิของหนูที่เพิ่มขึ้นได้อย่างมีนัยสำคัญทางสถิติ ส่วน DS, DL นั้นมีความแรงในการลดไข้น้อยมาก จากผลการทดลองแสดงว่า BMY ทุกขนาดที่ทำการทดสอบมีฤทธิ์ลดไข้ ซึ่งฤทธิ์ลดไข้ของ BMY อาจเกิดจากฤทธิ์ลดไข้ของ AM, OI และ WT การทดลองครั้งนี้เป็นการศึกษาแรกที่ใช้อธิบายฤทธิ์ทางเภสัชวิทยาของตำรับยาเบญจมูลใหญ่ และให้ข้อมูลทางวิทยาศาสตร์เพิ่มเติมที่สนับสนุนการใช้ตำรับยาแพทย์แผนไทยนี้

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KEYWORDS: *AEGLE MARMELOS* / *OROXYLUM INDICUM* / *DIMOCARPUS LONGAN* / *DOLICHANDRONE SERRULATA* / *WALSURA TRICHOSTEMON* / BEN-CHA-MOON-YAI REMEDY / LPS-INDUCED FEVER

SOMKIT BANSUTTEE: ANTIPYRETIC EFFECT OF FIVE ROOT EXTRACTS OF BEN-CHA-MOON-YAI REMEDY IN RATS. ADVISOR: ASST. PROF. FLG. OFF. PASARAPA TOWIWAT, Ph.D., CO-ADVISOR: ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., 138 pp.

Ben-Cha-Moon-Yai herbal remedy is an antipyretic and anti-inflammatory drug in Thai traditional medicine which includes roots of Ma-tum, Phe-ka, Lam-yai, Chare-tare and Kad-lin. We determined the antipyretic activity of the root extracts of Ben-Cha-Moon-Yai remedy (BMY), *Aegle marmelos* (AM), *Oroxylum indicum* (OI), *Dolichandrone serrulata* (DS), *Dimocarpus longan* (DL) and *Walsura trichostemon* (WT) using lipopolysaccharide (LPS)-induced fever model in rats compared to that of acetylsalicylic acid (ASA). Fever was induced in animals with an intramuscular injection of LPS (50 μg/kg) 1 hr after oral administration of 2% Tween 80, ASA 300 mg/kg or BMY (125-500 mg/kg) and various doses of AM, OI, DL, DS, WT (25, 50, 100, 200 and 400 mg/kg). Rectal temperature was measured before the pretreatment and at 1 hr intervals for 7 hr after LPS injection. All doses of BMY, AM and OI at the dose of 400 mg/kg and WT at the dose of 100 mg/kg significantly ($p<0.05$) attenuated the increased rectal temperature produced by LPS. DS and DL showed negligible antipyretic potency. These results demonstrated that BMY at all doses tested possesses antipyretic activity. The antipyretic effect of BMY may be due to the antipyretic property of AM, OI and WT. This is the first study that helps clarifying the pharmacological action of this herbal remedy and provides additional scientific support for this Thai traditional medicine.

Department : Pharmacology and Physiology Student's Signature .....

Field of Study : Pharmacology Advisor's Signature .....

Academic Year : 2010 Co-advisor's Signature .....

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LIST OF ABBREVIATIONS

α	= alpha
β	= beta
κ	= kappa
µg/kg	= microgarm per kilogram
µl	= microlitter
/	= per
%	= percent
^{o}C	= celcius degree
AM	= *Aegle marmelos* root extract
ASA	= acetylsalicylic acid
AUC	= area under the curve
BMY	= Ben-Cha-Moon-Yai remedy
BW	= body weight
cm	= centimeter
CNS	= central nervous system
DL	= *Dimocarpus longan* root extract
DS	= *Dolichandrone serrulata* root extract
ED_{50}	= median effective dose
et al.	= et alii (and other)
g	= gram
g/kg	= gram per kilogram
sec	= second
hr	= hour
IC_{50}	= median inhibition dose
i.p.	= intraperitoneal
i.m.	= intramuscular
LPS	= lipopolysaccharide
m	= meter
mg/kg	= milligram per kilogram

mg/ml	= milligram per milliliter
min	= minute
ml/kg	= milliliter per kilogram
N	= sample size
ng/kg	= nanogram per kilogram
NOS	= nitric oxide synthase
NSAIDs	= non-steroidal anti-inflammatory drugs
NSS	= normal saline solution
OI	= *Oroxylum indicum* root extract
TNF-α	= tumor necrosis factor-alpha
WT	= *Walsura trichostemon* root extract
w/w	= weight by weight

CHAPTER I

INTRODUCTION

Background and Rationale

Fever is defined as the elevation of core body temperature above normal. For most people, a temperature of 98.6 ^{o}F (37 ^{o}C) is baseline (Dalal and Zhukovsky, 2006). Fever usually occurs in response to an infection or inflammation. However, many other causes are possible, including drugs, poisons, cancer, heat exposure, injuries or abnormalities to the brain, or disease of the endocrine system. Exogenous pyrogens, such as microbial surface components of the gram negative bacterial outer membrane lipopolysaccharide (endotoxin), evoke pyrexia most commonly through the stimulation of pyrogenic cytokines. Endogenous pyrogens involved in producing a highly regulated inflammatory response to tissue injury and infection are polypeptide cytokines. Pyrogenic cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor (TNF), and interleukin-6 (IL-6), are those that act directly on the hypothalamus to affect a fever response. These signals trigger the release of other mediators, most notably prostaglandin E_2 (PGE_2), in the region of the preoptic nuclei of the anterior hypothalamus (POAH). Preoptic neurons bearing E-prostanoid receptors alter their intrinsic firing rate in response to PGE_2, evoking an elevation in the thermoregulatory set point to reach the new temperature that is higher than baseline (Aronoff and Neilson, 2001).

The world most commonly used drugs for the treatment of pain and fever are acetaminophen (paracetamol) and non-steroidal anti-inflammatory drugs (NSAIDs) such as acetylsalicylic acid and ibuprofen. Although NSAIDs are used for the treatment of pain, fever and inflammation, they are associated with several side effects including nausea, vomiting, diarrhea, constipation, decreased appetite, rash, dizziness, headache, and drowsiness. The most serious side effects are renal failure, liver failure, ulcers, gastrointestinal bleeding and prolonged bleeding. Excessive use of acetaminophen can damage multiple organs, especially the liver and kidney. Long term use of these drugs in chronic condition could lead to an expensive cost. Therefore, many researchers are searching for new antipyretic drugs with higher efficacy and lower side effects from natural products including herbal plants.

There are several Thai herbal formulas that have been used as antipyretic agents including Chan-Tha-Lee-La, Pra-Sa-Chan-Dang and Ben-Cha-Lo-Ka-Wi-Chian. “Ben-Cha-Moon-Yai” is a herbal remedy used in Thailand as an antipyretic and anti-inflammatory. The formula is composed of five herbal roots in an equal part by weight including roots of *Aegle marmelos* (Ma-tum), *Oroxylum indicum* (Phe-ka), *Dimocarpus longan* (Lam-yai), *Dolichandrone serrulata* (Chare-tare) and *Walsura trichostemon* (Kad-lin). Many researches had been done to investigate various pharmacological effects of the extract of several parts of these five herbal plants including root, leaf and stem bark. Although Ben-Cha-Moon-Yai remedy is widely used by many traditional doctors in Thailand as an antipyretic and anti-inflammatory agent, there is no scientific data that support its use. Therefore, this study was designed to investigate the antipyretic effect of the whole extract of the Ben-Cha-Moon-Yai remedy and individual root extracts using lipopolysaccharide-induced fever model in rats in order to provide scientific evidence to support its use in Thai traditional medicine.

Purpose of the study

To investigate the antipyretic effect of the whole extract of Ben-Cha-Moon-Yai remedy in comparison with extracts from its five individual components.

Hypothesis

Antipyretic effect of the whole extract of Ben-Cha-Moon-Yai remedy is due to antipyretic property of some of its individual components.

Expected benefit and application

The knowledge obtained from this study may lead to the development of a new antipyretic agent from natural sources and provide scientific evidence to support the use of Ben-Cha-Moon-Yai remedy as an antipyretic agent.

Research design

Experimental research

Key words

Aegle marmelos

Oroxylum indicum

Dimocarpus longan

Dolichandrone serrulata

Walsura trichostemon

Ben-Cha-Moon-Yai remedy

LPS-induced fever

CHAPTER II

LITERATURE REVIEWS

Thermoregulation

The human body has the remarkable ability to maintain a relatively constant temperature, despite wide fluctuations in several variables, including ambient temperature, energy expenditure, and energy intake (Goldman, Cimpello and Khine, 2000). Body temperature varies during the day (circadian rhythm) with the peak occurring in the late afternoon (5:00 PM to 7:00 PM) and the trough early in the morning (2:00 AM to 6:00 AM). Normal body temperature varies from an approximate low of 36.48^{o}C (97.68^{o}F) in the morning to a high of 36.98^{o}C (98.58^{o}F) in the late afternoon. This circadian variation can differ significantly between individuals and can be as much as 1.3^{o}C (2.4^{o}F) or as little as 0.1^{o}C (0.2^{o}F). This rhythm is less prominent during the first few months of life, and becomes established by the second year of life. The mechanisms of circadian variation are unclear, but this pattern appears to be a tightly regulated process. Circadian variation in body temperature can persist even during febrile illnesses, although it is absent in patients with hyperthermia.

At the heart of thermoregulation is an integrated network of neural connections involving the hypothalamus, limbic system, lower brainstem, the reticular formation, spinal cord, and the sympathetic ganglia. An area in and near the rostral hypothalamus is also important in orchestrating thermoregulation. This region, the 'preoptic area', includes the preoptic nuclei of the anterior hypothalamus (POAH) and the septum. The POAH maintains mean body temperature around a set point. This thermoneutral set point temperature is modulated by the balanced activities of temperature-sensitive neurons. These neurons integrate afferent messages regarding core body and peripheral (skin) temperatures and evoke various behavioral and physiologic responses controlling heat production or dissipation (Aronoff and Neilson, 2001, Goldman et al., 2000).

Fever

Fever is a pathologic elevation of the normal body temperature; it is an active process and resists changes by the external environment. Fever describes a regulated rise in body temperature after an increase in the hypothalamic set point. Under the influence of the hypothalamus, physiologic and behavioral functions favoring heat production and heat retention are stimulated until arriving at a newly elevated set point temperature. Typical early behavioral changes prior to fever include seeking a warmer environment or adding clothing. Physiologic alterations include cutaneous vasoconstriction, shivering, and none shivering. Upon reaching the elevated set point of fever, an increase or decrease in core temperature will stimulate thermoregulatory mechanisms similar to those evoked at normal body temperature. In other words, normal thermoregulation modulates at this higher set point (Aronoff and Neilson, 2001).

Recent data suggest that, while the heat of fever may kill some pathogenic microbes, more importantly it serves an adjuvant function by enhancing the effectiveness of certain selective, adaptive immune responses, thereby helping to compartmentalize the APR to the infected site. Benefits are provided in Table 1 (Blatteis, 2006).

Table 1 Some immune benefits of fever

- Enhanced neutrophil and monocyte motility and emigration
- Enhanced phagocytosis and pinocytosis
- Increased oxygen radical production by phagocytes
- Increased interferon (IFN) production
- Increased antiviral, antitumor, antiproliferative, and natural killer (NK)cell-
- Stimulating activities of IFN
- Potentiated IFN-induced anti-anaphylaxis (anergy)
- Enhanced expression of F_c receptors
- Increased T-helper cell activation, expression, recruitment, and cytotoxic activity
- Increased antibody production
- Increased T-cell proliferative response to non specific mitogens, IL-1 and -2, and allogeneic lymphocytes

- Increased killing of intracellular bacteria
- Increased bactericidal effect of antimicrobial agents
- Induction of cytoprotective heat shock proteins (HSPs) in host cells
- Induction of pathogen HSPs, which activate host defenses

Fever, in this context, provides the optimal thermal environment for the timely and appropriate expression of the various factors that, in concert, constitute the most effective antimicrobial host defense.

Hence, anti-pyretic medications, by defeating the very purpose of this precisely patterned and optimized host defense response, should be avoided unless overriding conditions exist (Table 2), and letting fever take its natural course should be the preferred approach, at least early on during an infection's course. And, should anti-pyretic interventions be indicated, the temporal relationship between the expressions of the various pro-and anti-inflammatory mediators induced in response to the infection reviewed earlier should be taken into account when considering the timing and type of specific anti-pyretic treatments (Blatteis, 2006).

Table 2 Some hazardous effects of high or prolonged fever

A. Potentially harmful effect	B. High-risk predisposing factors
Dehydration	Deteriorated, malnourished state
Delirium	Precarious water balance
Localized lesions (e.g., liver, brain)	Cardiorespiratory disease
Convulsions	Craniocerebral trauma or disease
Cardiopulmonary strain	Epileptic lesions
Negative nutrient balances	Severe mental disorder
Teratological consequences	Pregnancy
	Host defense defects

Pathology of fever

Many of the mediators underlying pyrexia have been described in recent years (Figure 1). The critical 'endogenous pyrogens' involved in producing a highly regulated inflammatory response to tissue injury and infections are polypeptide cytokines.

Pyrogenic cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor (TNF-α), and interleukin-6 (IL-6), are those that act directly on the hypothalamus to effect a fever response. Exogenous pyrogens, such as microbial surface components, evoke pyrexia most commonly through the stimulation of pyrogenic cytokines. The gram-negative bacterial outer membrane lipopolysaccharide (endotoxin), however, is capable of functioning at the level of the hypothalamus, in much the same way as IL-1β. These signals trigger the release of other mediators, most notably prostaglandin E (PGE), in the region of the POAH. PGE_2 is believed to be the proximal mediator of the febrile response. Preoptic neurons bearing E-prostanoid receptors alter their intrinsic firing rate in response to PGE, evoking an elevation in the thermoregulatory set point. There are four known cellular receptors for PGE_2: EP_1 through EP_4. The particular receptor subtype involved in pyrogenesis is unknown. Although mice lacking the neuronal PGE_2 receptor subtype EP_3 demonstrate an impaired febrile response to both exogenous (endotoxin) and endogenous pyrogens, studies in rats appear to implicate the EP_4 receptor. The intracellular events triggering pyrexia after PGE-EP receptor coupling among species are unclear.

Fever is tightly regulated by the immune response. Inflammatory stimuli triggering the generation of propyretic messages provoke the release of endogenous antipyretic substances. Substances such as arginine vasopressin (AVP), α-melanocyte stimulating hormone, and glucocorticoids act both centrally and peripherally to limit pyrexia. The cytokine interleukin-10 (IL-10) has numerous anti-inflammatory properties, including fever suppression. In addition, a class of lipid compounds known as epoxyeicosanoids generated by certain cytochrome P-450 enzymes plays an important role in limiting the fever and inflammation. Analogous to a biochemical feedback pathway, fever itself appears capable of countering the release of pyrogenic cytokines. For example, febrile temperatures augment early TNF release in endotoxin challenged mice, yet limit its prolonged (and perhaps detrimental) expression after either lipopolysaccharide injection or bacterial infection (Figure 1; Aronoff and Neilson, 2001).

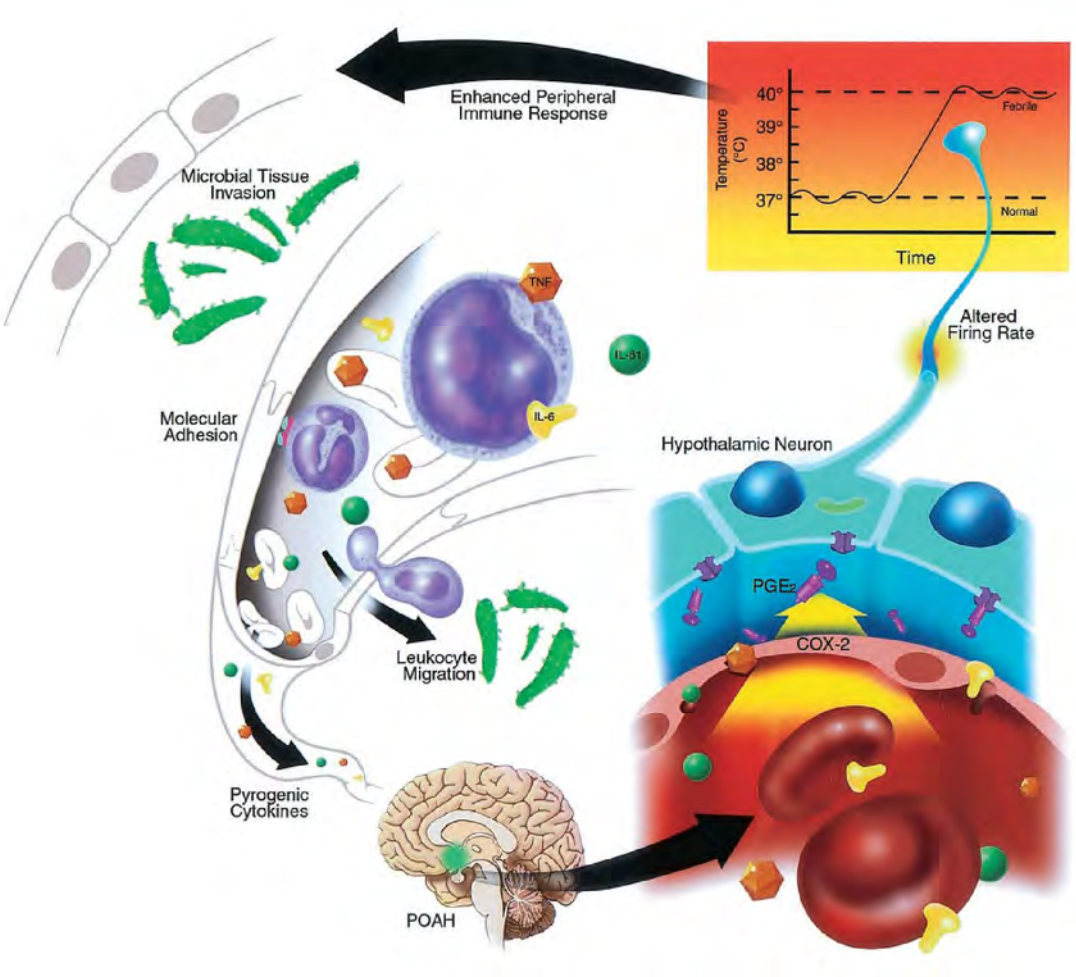

Figure 1 The pathogenesis of fever (Aronoff and Neilson, 2001).

The role of exogenous pyrogens

Many different substances, microbial and non-microbial, are capable of providing this pyrogenic stimulus (Table 3). Since they originate outside the body, they are called exogenous pyrogens. Mostly, these are the invading infectious microbes or their products recognized as foreign by specific, evolutionarily conserved germ-line-encoded patterns, termed pathogen-associated molecular patterns (PAMPs), that they secrete or are carried on their surface. These PAMPs are detected by equally conserved, one-pass transmembrane receptors, called toll-like receptors (TLRs), present on the invaded host's immune cells. Their recognition by myeloid cells at the outset of an infectious challenge is the trigger that entrains the complex interactions of signals that eventuate in the activation of the innate immune response, including fever. For example, PAMPs produced by cell-wall components of gram-positive bacteria (peptidoglycan,

lipotei-choic acid) are recognized by TLR2; of gram-negative bacteria (lipopolysaccharide, LPS) by TLR4; flagellin, the major element of bacterial flagella, by TLR5 and bacterial DNA by TLR9. The double-stranded DNA (dsRNA) produced by viruses is recognized by TLR3 (Blatteis, 2006).

Table 3 Some common pathogenic stimuli (exogenous pyrogens) that induce fever

A. Microbial	
Viruses	Whole organism, hemagglutinin, dsRNA
Bacteria	Gram-positive – whole organisms, peptidoglycans (e.g., muramyl dipeptide, lipoteichoic acids, exotoxins, enterotoxins, erythrogenic toxins, group B polysaccharides Gram-negative – whole organisms, peptidoglycans, lipopolysaccharides [lipid A]
Mycobacteria	Whole organisms, peptidoglycans, polysaccharides, lipoarabinomannan)
Fungi	Whole yeasts, capsular polysaccharides, proteins
B. Non-microbial	
Antigens	e.g., Bovine or human serum albumin, bovine gamma globulin, ovalbumin, penicillin
Inflammatory agents	e.g., Asbestos, silicia, uv radiation, turpentine
Plant lectins	e.g., Concanavalin A, phytohemagglutinin
Drugs	e.g., Polynucleotides (e.g., polyriboinosinic:polyribocytidylic acid), anti-tumor agents (e.g., bleomycin), plant alkaloids (e.g.,colchicines), synthetic immunoadjuvants (e.g., muramyl peptides)
Host-derived	e.g., Antigen– antibody complexes, activated complement fragments, inflammatory bile acids, urate crystals, certain androgenic steroid metabolites (e.g.,etiocholanolone), certain lymphocyte product

The role of pyrogenic cytokines (endogenous pyrogen)

Interleukin-1 (IL-1β)

The members of the IL-1 family, IL-1beta, IL-1alpha and the receptor antagonist IL-1ra, are probably the most extensively investigated cytokines. IL-1alpha, IL-1beta and IL-1ra all bind the same IL-1 receptors: the type I and type II IL-1 receptors. The type I IL-1 receptor (IL-1RI) is a member of the Toll receptor family. The heterodimeric IL-1RI complex is composed of the IL-1 binding protein receptor (IL-1R) and the IL-1R accessory protein (IL-1RAcP) responsible for signal transduction. Binding of IL-1 to the IL-1RI leads to structural changes allowing docking of the IL-1R-AcP, a necessary event for the recruitment/activation of the myeloid differentiation primary response protein (MyD88), IL-1 receptor-associated kinase (IRAK), TNF receptor-associated factor (TRAF) signaling pathway ultimately leading to activation of the nuclear factor kappa B (NF-κB) (Bruno, 2004). The most convincing experiment that IL-1 was pyrogenic was the induction of a rapid-onset fever in mice, rabbits, rats, guinea pigs and humans following a single injection of the recombinant human or other mammalian IL-1, either IL-1β or IL-1α. Since recombinant cytokines are expressed in *Escherichia coli*, considerable caution needed to be employed to exclude the involvement of contaminating bacterial endotoxins in the recombinant preparations. Endotoxin evokes fever in rabbits at concentrations as low as 10 ng/kg and in humans even lower doses of 1 ng/kg are pyrogenic (Dinarello, 2004).

Interleukin-6 (IL-6)

Interleukin 6 (IL-6) is an important mediator of the host response to disease. IL-6 administration in humans causes fever but unlike IL-1, microgram/kilogram doses are needed compared to nanogram/kilogram for IL-1. There have been multiple reports of elevated IL-6 levels in a variety of human diseases and in various human body fluids such as plasma, cerebrospinal fluid and joint fluids. IL-6 gene expression is, in part, under the control of several exogenous pyrogens but also IL-1 and TNF. In fact, IL-1 and particularly IL-1 plus TNF are very potent stimulators of IL-6 gene expression and protein translation. Therefore, IL-6 is often elevated in conditions where IL-1 and TNF-α have been synthesized (Dinarello, 2004).

Tumor necrosis factor-alpha (TNF-α)

The tumor necrosis factor alpha (TNF- α or TNF) is the principal mediator of acute inflammation in response to gram-negative bacteria. It is mainly produced by LPS-activated mononuclear phagocytes, but can be secreted also by antigen-stimulated T cells, natural killer and mast cells. Serum TNF levels rise during the initial phase of the LPS-induced hypothermia (Conti et al., 2004). At 100–200 ng/kg, recombinant human TNF-α produces the typical endogenous pyrogen-fever pattern in rabbits and C3H/HeJ mice. The rapid rise in body temperature that occurs in rabbits after an intravenous injection of TNF-α is indistinguishable from that produced by either form of recombinant IL-1, albeit at 10-fold lower doses. TNF-α evokes a second fever peak 3–4 h after the injection. Plasma taken from rabbits during the second fever peak contains circulating IL-1; this finding formed the basis of the concept that TNF-α induces IL-1 *in vivo* (Dinarello, 2004).

Prostaglandins (PGs)

Interest in these compounds dates back to the early 1970s when it was found that PGE_1 was a potent pyretic agent and that antipyretics blocked prostaglandin synthesis in various organs including brain. These two findings implicated a prostaglandin in the genesis of fever. Research in this area developed actively and led to the demonstration that: (a) PGE_2, a normal constituent of hypothalamic tissue, is as potent as PGE_1 in producing fever moreover, both compounds are like pyrogens in that their action is not influenced by ambient temperature; (b) PGE_2 acts upon neurons in the POAH that are also the main target for pyrogens; (c) thermo-sensitive neurons in POAH respond in the same manner to PGE_2 and pyrogens; (d) PGE_2 fever, unlike pyrogen fever, does not abate following administration of antipyretics; and (e) pyrogen fever is associated with elevated levels in the CSF of a prostaglandin with the biological and immunological properties of PGE. Collectively, these findings indicate that PGE is well suited for being the 'central messenger' of fever and specifically of pyrogen fever. According to current knowledge, pyrogens from outside the body (exogenous pyrogen), and foremost among them bacterial endotoxin, as well as pathological conditions causing tissue inflammation and damage (e.g. infarction, malignancy) elicit the formation of a pyrogenic substance (endogenous pyrogen) in neutrophils and in cells of

then reticuloendothelial system. The endogenous pyrogen, which is therefore a key intermediate in the sequence of events leading to fever, is then carried to the rostral region of the hypothalamus by the circulation. Because the blood-brain barrier is seemingly impermeable to endogenous pyrogen, and because prostaglandins are rapidly removed from the circulation, one must assume that the vessel wall is the main site where pyrogen action is translated into increased prostaglandin synthesis. Consistent with this hypothesis is the notion that vessels, including cerebral vessels, are endowed with an active prostaglandin-generating system and that hypothalamic blood flow is increased during pyrogen fever. The latter finding implies activation of prostaglandin synthesis in the vessel wall. Alternatively, PG could be released from phagocytosing leukocytes sequestered in the capillary bed of POAH. Any pyrogen crossing the blood-brain barrier may stimulate prostaglandin synthesis in neural tissue. PGE, whether formed in the tissue of the POAH or from the vessels, acts at appropriate sites in the thermoregulatory pathways to elevate the 'set-point' for temperature regulation, thus causing fever. Once its action is completed, PGE_2 is either inactivated enzymatically *in situ* or enters the extracellular fluid and CSF when it is transported into the circulation. Interference with the latter mechanism results in enhancement of pyrogen effects. PGE_2 is synthesized from arachidonic acid, which is released from cell membrane lipid by phospholipase. Arachidonic acid is metabolized by two isoforms of the cyclooxygenase (COX) enzyme, COX-1 and COX-2. COX-1 usually is expressed constitutively and generates prostanoids important to housekeeping functions supporting homeostasis. COX-2, on the other hand, is inducible by inflammatory signals such as the pyrogenic cytokines, IL-1β, TNF-α, and IL-6, and bacterial lipopolysaccharide. Genetically engineered mice that lack either the COX-1 or COX-2 gene demonstrate that the inducible isoform is responsible for hypothalamic PGE_2 production during a febrile response. As COX-2 is the key provider of PGE_2 during pyrexia, it is not surprising that the selective COX-2 antagonist is an effective antipyretic in humans. Many cells, including synoviocytes, macrophages, endothelial cells, and chondrocytes, have the capacity to rapidly up-regulate the expression of the COX-2 during inflammation. The most likely cell type in the central nervous system responsible for producing PGE_2 is the microvascular endothelial cell, which expresses

COX-2 exuberantly after stress. An effective febrifuge might interrupt pyrexogenesis at any step that connects peripheral inflammation with the central production of PGE. Stated differently, an antipyretic might blunt peripheral inflammation or depress central pyrogenic signals, or affect both. Inhibiting central production of PGE_2 is a well-known mechanism of antipyretic agents, but activated leukocytes and endothelial cells in peripheral areas of inflammation also represent potential drug targets (Aronoff and Neilson, 2001).

Pharmacological methods of fever management

Antipyretic drugs

Acetaminophen

Acetaminophen is an analgesic that deserves special comment because it is an effective febrifuge but a weak anti-inflammatory drug. Its effects differ considerably from salicylates and other NSAIDs. As opposed to aspirin, acetaminophen is a poor inhibitor of platelet function. Believed to be an inhibitor of cyclooxygenase, acetaminophen's mechanism of action is still poorly understood. Although suprapharmacologic doses of acetaminophen inhibit NF-κB stimulation of inducible nitric oxide synthase, it does not possess the same inhibitory effects on NF-κB–mediated gene transcription that salicylates enjoy. Explanation of the antipyretic and analgesic actions of acetaminophen has been based on tissue-specific COX inhibition not seen with NSAIDs. Acetaminophen penetrates the blood-brain barrier, achieving cerebrospinal fluid levels comparable to those in serum, and may act preferentially within the central nervous system. Central nervous system levels of PGE_2 rise during fever and fall to normal levels upon administration of the drug. Acetaminophen reduces the production of prostaglandins in brain preparations more potently than it does from other organs such as spleen (Aronoff and Neilson, 2001).

Aspirin and NSAIDs

The antipyretic drug aspirin was in wide clinical use for more than 70 years before Vane demonstrated in 1971 that it exerted its physiologic action by inhibiting the production of prostaglandins. Further work suggests a current model of how aspirin and similar NSAIDs act as antipyretics. Aspirin interferes with the

biosynthesis of cyclic prostanoids derived from arachidonic acid, such as thromboxane A_2 and prostaglandins. As a nonselective COX inhibitor, aspirin has been widely studied for its anti-inflammatory, antipyretic, and antithrombotic traits. The major mechanism of action of aspirin and other antipyretics involves lowering PGE_2 by directly inhibiting COX enzyme activity. NSAIDs are also capable of reducing PGE_2 production by down-regulating the expression of COX enzymes, as opposed to directly inhibiting their enzymatic action. Sodium salicylate and aspirin also inhibit COX-2 transcription induced by lipopolysaccharide and IL-1β. The clinical effects of sodium salicylate are likely due in part to its actions on COX gene transcription by disabling the transcriptional activator nuclear factor-κB (NF-κB). NF-κB is a heterodimeric protein capable of binding DNA in the 5'-promoter regions of many genes involved in the inflammatory response. Once bound, NF-κB facilitates the transcription of genes encoding pyrogenic cytokines, chemokines, adhesion molecules, and inflammatory enzymes, including inducible nitric oxide synthase and COX-2 in certain cell types. NF-κB resides in an inactive state in the cytoplasm, complexed to another protein, IκB. Upon activation, the IκB silencer is sequentially phosphorylated, ubiquinated, and degraded, releasing NF-κB to translocate into the nucleus. Salicylates reduce the nuclear translocation of NF-κB through stabilization of cytoplasmic IκB by interfering with its phosphorylation. The ability of antipyretics to disable transcription varies among agents and cell type studied. Salicylate and its progenitor aspirin prevent NF-κB translocation in endothelial cells and leukocytes induced by proinflammatory cytokines or lipopolysaccharide. NSAIDs like ibuprofen also block the nuclear trafficking of NF-κB in certain tumor cell lines but fail to do so in activated macrophages. Indomethacin, another COX inhibitor, does not appear to affect NF-κB, and therapeutic doses of acetaminophen also fail to suppress it. The reason for this heterogeneity is unknown (Aronoff and Neilson, 2001).

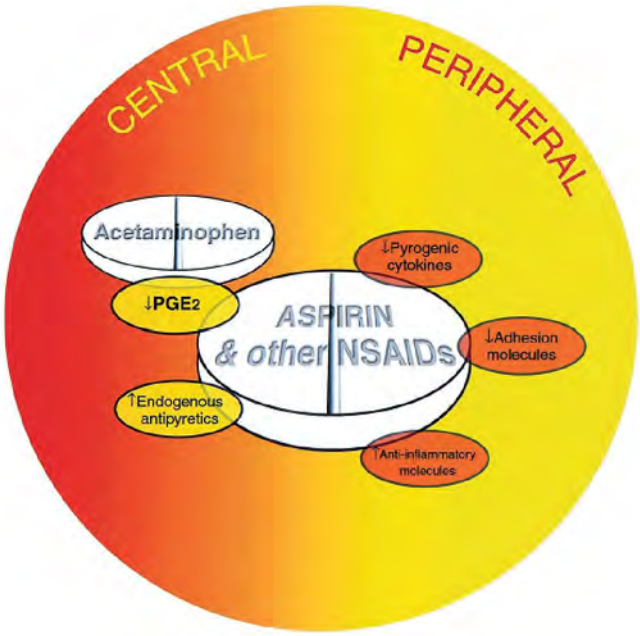

Figure 2 Mechanisms of antipyretics (Aronoff and Neilson, 2001)

Ben-Cha-Moon-Yai Remedy

Ben-Cha-Moon-Yai remedy is one of the antipyretic herbal remedies in Thai traditional medicine. It is widely used by traditional doctors in Thailand to treat many types of fever and inflammation without any scientific data support. Ben-Cha-Moon-Yai remedy is composed of five herbal roots in an equal part by weight, including roots of *Aegle marmelos* (Ma-tum), *Oroxylum indicum* (Phe-ka), *Dimocarpus longan* (Lam-yai), *Dolichandrone serrulata* (Chare-tare) and *Walsura trichostemon* (Kad-lin). Many studies have shown that all those plants contain many alkaloids, sterols and other compounds. Until now, no work has been done to demonstrate the antipyretic effect of Ben-Cha-Moon-Yai remedy scientifically. The only scientific evidence that may support its use as an antipyretic drug came from a work of Arul et al. in 2005 that reported the anti-inflammatory, antipyretic and analgesic properties of the serial extracts of leaves of *Aegle marmelos,* one herbal plant in the Ben-Cha-Moon-Yai remedy.

Aegle marmelos (Linn.) Corr.

Figure 3 *Aegle marmelos* (Linn.) Corr. fruit and *Aegle marmelos* (Linn.) Corr. root

Family Rutaceae

Vernacular names

Thailand: matum, tum (Pattani), ma pin (north). Bael or bel fruit (En). Bel Indien (Fr). Indonesia: maja, maja batu. Malaysia: bilak, bila, bel. Philippines: bael. Burma: opesheet, okshit. Cambodia: bnau. Laos: toum. Vietnam: trái mam.

Distribution

Bael grows wild in dry forests in the Indian Peninsula, Sri Lanka, Pakistan and Bangladesh. It is an old cultivated tree in that region, particularly found in temple gardens in India. It has spread to Indo-China, South-East Asia (in particular Thailand, northern Malaysia, eastern Java and northern Luzon) and other parts of the tropics (Sunarto, 1991).

Uses

Ripe fruit is eaten fresh and is also prepared as a sherbet, syrup, marmalade and fruit nectar. The mucilage around unripe seeds is used as adhesive and household glue. The extract of leaf and young fruit was used in Java to adulterate opium. In Java the nearly ripe fruit is sliced, dried and applied against chronic dysentery, diarrhea and constipation. Ripe fruit extract is also used against rectum inflammation. The rind of unripe fruit can be used as a yellow dye and as a tanning agent.

In Indo-China bark and leaves are used against intermittent fever, but in Sulawesi the bark is used to poison fish. Young leaves are used for seasoning in Java,

although one source says they may cause abortion and sterility in women; together with betel pepper and lime they are rubbed on itching skin and used as poultice for wounds. In Madura the leaf juice is used against foot-and-mouth disease in cattle.

The root is used against heart palpitation, indigestion and bowel inflammation (Sunarto, 1991).

Properties

The pulp is soft, yellow or orange, very fragrant and pleasantly flavored. The edible portion (pulp) amounts to 56-77% of the fruit and contains per 100 g: water 61.5 g, protein 1.8 g, fat 0.39 g, carbohydrates 31.8 g, ash 1.7 g, carotene 55 mg, thiamine 0.13 mg, riboflavin 1.19 mg, niacin 1.1 mg, and vitamin C 8 mg. The fruit is rich in tannin (up to 20% in the rind). Marmelosine ($C_{13}H_{12}O_3$), volatile oil, limonene, alkaloids, coumarines and steroids are also present in different parts of the tree (Sunarto, 1991).

Phytochemicals

tannin, skimminanin, essential oil, sterol, triterpenoids, lupeol, sitosterol, amyrin, flavanoid, aegelin, marmesin, umbelliferone

Pharmacological Activities

Analgesic, Anti-inflammatory and Antipyretic Activity:

Arul et al. (2005) demonstrated anti-inflammatory, antipyretic and analgesic properties of the serial extracts of the leaves of *Aegle marmelos*. Anti-inflammatory property was assessed utilizing carrageenan-induced paw edema and cotton-pellet granuloma in rats. Antinociceptive activity was assessed utilizing acetic acid-induced writhing in mice and antipyretic activity was assessed utilizing yeast-induced hyperpyrexia in mice. Similarly, Ghangale et al. (2008) also evaluated anti-inflammatory activity of the aqueous extract of *A. marmelos* using rat paw oedema model and proposed that *A. marmelos* possesses anti-inflammatory activity. Shankharananth et al. (2007) demonstrated that the methanolic extract of the leaves of *A. marmelos* at a dose level of 200 and 300 mg/ kg possesses significant analgesic activity on acetic acid-induced writhing and tail flick test in mice.

Antidiabetic Activity:

The aqueous extract of A. *marmelos* leaves was evaluated for hypoglycemic and antioxidant effects by Upadhya et al. (2004) using alloxan-induced diabetes in male albino rats and proposed that the aqueous extract may be useful in the long-term management of diabetes. Similarly, the antihyperlipidemic activity of the aqueous extract of *A. marmelos* fruits was demonstrated by Marinzene and Gilbart (2005) using the streptozotocin-induced diabetic Wistar rats. Sundaram et al. (2009) worked on the alcoholic extract of *A. marmelos*, *Momordica charantia* and *Eugenia jambolana* against streptozotocin-induced diabetic rats and confirmed their protective activity against laboratory induced cell necrosis. Where as, Kuttan and Sabu (2004) studied on the leaf extract of *A. marmelos* on alloxan-induced diabetes and reported that the extract was capable of reducing oxidative stress by scavenging lipid peroxidation and enhancing certain antioxidant levels which causes lowering of elevated blood glucose level. Beside of all above cited work, Hema and Lalithakumari (1999) had presented a tremendous result of *A. marmelos* and documented its hypoglycemic action along with other pharmacological actions on molecular level.

Hepatoprotective Activity:

Singanan et al. (2007) worked on *A. marmelos* leaf extract on alcohol-induced liver injury in albino rats and presented data of excellent hepatoprotective effects. Similarly, Singh and Rao (2008) also demonstrated that the aqueous extract of bael fruit pulp and seeds are effective in the treatment and prevention of CCl_4- induced hepatic toxicity.

Antimicrobial Activity:

Maheshwari et al. (2009) studied on the ethnolic extract of dried fruit pulp of *A. marmelos* against various intestinal pathogens i.e. *Shigella boydii*, *S. sonnei* and *S. flexneri* and proposed that certain phytochemicals including phenols, tannins and flavonoids were effective against all. It was also confirmed by Kaur et al. (2009) by treating *E. coli* with *A. marmelos* fruit extract. In consonance, Citarasu et al. (2003) also performed some experiments with *A. marmelos* on certain pathogenic bacteria like *Salmonella typhi*, *Pseudomonas aeruginosa*, *Aeromonas hydrophyla* and *Vibrio sp*., and concluded its positive bactericidal effects.

Antifungal Activity:

Patil, Chaudhary and Settipalli (2009) reported the antifungal, antidiarrheal and antimicrobial activities of the ethanolic extract of *A. marmelos* leaves. Rana, Singh and Taneja (1997) evaluated the antifungal activity of essential oils isolated from the leaves of Bael using spore germination assay. The oil exhibited variable efficacy against different fungal isolates and 100% inhibition of spore germination of all the fungi tested was observed at 500 ppm. They proposed that the essential oils from bael leaves may interfere with the Ca^{2+}-dipicolonic acid metabolism pathway and possibly inhibit the spore formation. Pitre and Srivastava (1987) demonstrated the antifungal activity of the ethanolic root extract against *Aspergillus fumiganus* and *Trichphyton mentagrophytes*.

Anticancer Activity:

Latica and Costa (2005) evaluated the anticancer potential of folk medicine used in Bangladeshi and tested the extracts of *A. marmelos* for cytotoxic action using brine shrimp lethality assay, sea urchin eggs assay and MTT assay using tumor cell lines. The extract of *A. marmelos* was found to exhibit toxicity on all used assays. Similarly, Jagetia, Venkatesh, and Baliga (2005) reported the anticancer effect of the hydroalcoholic extract of bael leaves in the animal model of Ehrlich ascites carcinoma and proposed that the induction of apoptosis may be due to the presence of skimmianine in the extract.

Radioprotective Activity:

Radioprotective effect of *A.marmelos* extract was studied by Jagetia and Venkatesh (2005) by exposing mice to different doses of gamma-radiation and found that oral administration of the extract resulted in an increase in radiation tolerance by 1.6 Gy. Again, Jagetia et al. (2006) studied effects of the plant extract on the peripheral blood and small intestine of Swiss albino mice. They exposed the animals to gamma radiation and data were collected against radiation-induced changes in the peripheral blood, spleen colony forming units, and intestinal mucosa. They reported that *A. marmelos* extract significantly reduces the deleterious effect of radiation in intestine and bone marrow of mouse.

Antispermatogenic Activity:

Pramanik et al. (1999) reported antispermatogenic acitivity of the ethanolic extract of *A. marmelos* leaves in rats. Again, the same workers, including Sur et al. (2002) presented data of antimotility of rat sperms through *in vitro* study. Similarly, Sharma et al. (2009) studied the effect of the ethanol extracts of leaves of *A. marmelos* for their *in vitro* effect on sperm motility and suggested that the extracts had a considerable effect on the motility of sperm. It was also proposed that an increase in concentration of the extracts decreased the motility of sperms.

Antiulcer Activity:

Goel et al. (1997) reported that oral administration of pyranocoumarin isolated from the seeds of *A. marmelos* showed significant protection against pylorus-ligated and aspirin-induced gastric ulcers in rats and cold restraint stress-induced gastric ulcers in rats and guinea pigs. Dhuley (2007) reported that pretreatment of rats with unripe bael fruit extract produce a significant inhibition of absolute ethanol-induced gastric mucosal damage.

Antithyroid Activity:

Panda and Kar (2006) isolated scopoletin (7-hydroxy-6-methoxy coumarin) from *A. marmelos* leaves and evaluated for its potential to regulate hyperthyroidism. It was observed that oral administration of scopoletin (1.00 mg/kg for 7 days) to levo-thyroxine treated animals decreased serum thyroid hormones level. It was also proved that scopoletin has superior therapeutic activity than the standard antithyroid drug, propylthiouracil.

Toxicity Studies:

Total alcoholic, total aqueous, whole aqueous and methanolic extracts were collected from the leaves of *A. marmelos* by Veerappan et al. (2007) and studied in experimental rats for their toxicity. No histopathological changes were found when the extracts of *A. marmelos* were administered intraperitoneally for 14 days at the dose of 50 mg/kg body weight. The collected data demonstrated that the extracts of the leaves of *A. marmelos* have a high margin of drug safety.

Oroxylum indicum (Linn.) Kurz.


Figure 4 *Oroxylum indicum* (Linn.) Kurz. fruit and *Oroxylum indicum* (Linn.) Kurz. root

Family Bignoniaceae

Synonyms

Bignonia indica L. var.'ALFA'a (1753), *Bignonia pentandra* Lour. (1790), *Calosanthes indica* (L.) Blume(1826).

Vernacular names

Midnight horror (En). Indonesia: pongporang (Sundanese), kayu lanang, mungli (Javanese). Malaysia: beka, bonglai, kulai. Philippines: pingka-pingkahan (Tagalog), abong-abong (Bisaya), kamkampilan (Iloko). Cambodia: pi ka. Laos: lin may, ung ka. Thailand: phe kaa (central), litmai (northern), lin faa (north-eastern).

Distribution

Oroxylum indicum is found from India eastward to southern China and the Philippines, and throughout South-East Asia; in Indonesia eastward to Sulawesi and the Lesser Sunda Islands. Locally cultivated near human settlements.

Uses

Throughout its distribution area, the bitter bark is employed for intestinal complaints. It is credited with astringent and tonic properties, and widely used for diarrhea and dysentery. In Java, the pounded bark mixed with water is taken in gastritis and to purify the blood. In northern Sulawesi, the inner bark is used to arrest bleeding.

In Malaysia, a decoction of the leaves is drunk for stomach-ache. Externally it is employed in cholera, fever, childbirth and rheumatic swellings. The boiled leaves are employed as a poultice during and after childbirth, and in dysentery as well as for an enlarged spleen. Leaf poultices may be further applied for toothache and headache.

In the Philippines, a decoction of the root is credited with antirheumatic, antidysenteric and diuretic properties; the leaves are used in antirheumatic baths. In Thailand, the root and root bark are used for diarrhea and dysentery, while the stem bark is applied for ulcers and abscesses.

In Vietnamese folk medicine, a decoction of the seeds is used for cough, bronchitis and gastritis. Externally the seeds are applied to ulcers. A decoction of the dried root bark or stem bark is used in the treatment of allergic diseases, urticaria, jaundice, asthma, sore throat, laryngitis, hoarseness, gastralgia, diarrhea and dysentery. An alcoholic maceration of the fresh bark is externally applied on allergic dermatitis. In Thai folk medicine, the root is employed as a tonic and antidiarrheal, whereas the seed is used as a laxative and expectorant.

Throughout South-East Asia, cooked flowers, buds and young pods are highly esteemed as a vegetable. In Java, flowers, young shoots and the stem bark are consumed fresh as a side dish. The wood can be used as firewood although it is of poor quality.

Observation

A semi-deciduous, sparingly branched tree up to 27 m tall; trunk up to 40 cm in diameter, bark grey, with prominent leaf scars, twigs thick, pithy, later hollow, lenticellate. Leaves crowded, imparipinnate, 3-4 times pinnate, 0.5-2 m long; petiole long, rachis swollen at points of insertion; stipules absent; leaflets ovate to oblong, 4-11(-15) cm x 3-9 cm, base cuneate or mostly oblique, apex acuminate, entire, with scattered glands on the lower surface. Inflorescence an erect raceme, terminal, 25-150 cm long, peduncle and rachis partitioned.

Flowers bisexual, pedicel 2-4 cm long, bracteolate; calyx coriaceous, campanulate, containing water in bud, 2-4 cm long, 1.5-2 cm in diameter, brown or dirty violet, becoming almost woody in fruit; corolla funnel-shaped, about 10 cm long, lobes 5, subequal, margin wrinkled, reddish outside, yellowish to pinkish inside; stamens 5, inserted in the throat, hairy at the base; ovary superior, 2-celled, many-ovuled.

Fruit a pendent capsule, sword-shaped, 45-120 cm x 6-10 cm, valves flat, almost woody, finally black.

Seed 5-9 cm x 2.5-4 cm, including the membranous and transparent wing. Seedling with epigeal germination; hypocotyl elongated; cotyledons leafy.

Properties

The various parts of *Oroxylum indicum* are rich in flavonoids. The leaves contain the flavonoids baicalein (5,6,7-trihydroxyflavone), scutellarein (4',5,6,7-tetrahydroxyflavone), and their glycosides baicalin (baicalein-7-glucuronide) and scutellarin (scutellarein-7-glucuronide). The stem and root bark contain e.g. baicalein, scutellarein, oroxylin A (5,7-dihydroxy-6-methoxyflavone), chrysin (5,7-dihydroxyflavone) and p-coumaric acid. Baicalein and oroxindin (wogonin-7-O-'BETA'-D-glucuronide) have been isolated from the seeds. Other compounds mentioned in the literature include the prenylated naphthoquinone lapachol, and the anthraquinone derivative aloe-emodin.

The isolated flavonoid baicalin also showed inhibitory effects against the human T cell leukaemia virus type 1, and the human immunodeficiency virus (HIV-1). Baicalein furthermore showed antiproliferative activity in cultured rabbit vascular muscle cells, and lipoxygenase activity *in vitro*. Other pharmacological activities of baicalin and baicalein include anti-inflammatory activity of baicalin in the rat adjuvant arthritis model, inhibition of LPS-induced IL-1 production by both flavonoids, and inhibition by baicalein of leukotriene C-4 biosynthesis by rat resident peritoneal macrophages. The methanol extract of the young perianth exhibited strong antitumour-promoting activity when tested against 12-O-tetradecanoylphorbol-13-acetate (TPA) induced Epstein - Barr virus early antigen activation (Rasadah, 2001).

Pharmacological Activities

Antimicrobial Activity:

Dichloromethane extracts of the stem bark and root of *Oroxylum indicum* were found to have antimicrobial activities against gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and yeast (*Candida albicans*).

Bioassay-guided chromatographic fractionation led to the isolation of flavonoids (e.g. baicalein, chrysin and oroxylin A) and lapachol as active constituents. Lapachol was found to be active against the gram-positive bacteria; 5 µg gave a zone of inhibition equivalent to that shown by 5 µg of streptomycin, whereas 5 µg of chrysin gave

inhibition zones of equal size to that of 5 µg of streptomycin against *Pseudomonas aeruginosa* (Ali et al., 1998). The natural product, chrysin (5,7-dihydroxflavone), obtained from *O. indicum*, were screened for antibacterial activity against a panel of susceptible and resistant gram-positive and gram-negative organisms. It was observed that most of the derivatives displayed significant activity (Babu et al., 2006)

Anti-inflammatory and Antiallergic activities

A liquid extract of bark lowered the vascular permeability and suppressed the inflammatory edema of rats sensitized with egg protein, formalin or histamine. This extract had no effect on vascular permeability of rats sensitized with horse serum or xylene (dimethylbenzene). The anti-inflammatory effect of this preparation was more pronounced in the sensitized animal than in the normal ones (Golikov and Brekhman, 1967).

A lipophilic extract of *O. indicum* stem bark gave 100% inhibition of leukocyte lipoxygenase at a concentration of 50 g/ml which was mainly due to lapachol. The inhibitory activity of lapachol from *O. indicum* root against soya bean 5-lipoxygenase (IC_{50} 0.79 µg/ml) was equivalent to that of the positive control (the flavonoid fisetin; IC_{50} 0.97 µg/ml), whereas 50 µg/ml of the dichloromethane extract of the root bark gave 100% inhibition of leukocyte lipoxygenase. These activities might indicate an anti-inflammatory effect for the dichloromethane extract, mainly due to its lapachol content (Ali et al., 1998).

Antimalarial activity:

The ethanol-water (1:1) extract of dried bark showed antimalarial activity against *Plasmodium falciparum*, but a water extract had no effect (Suppakun et al., 1982). However, the data form these studies were incomplete. The extract (unspecified solvent) from stem bark had an *in vitro* response against *P. falciparum* at a MIC over 25 µg/ml (Dechatiwongse Na Ayudhya et al., 1989). A clinical trial on the antimalarial activity of bark was carried out at a dose of 15-30 g of the ethanol extract per day; 67% of the patients were claimed to be cured (Ketsingh, 1950).

Antimutagenic Activity:

The antimutagenic activity of the methanolic extract of fresh fruit of *O. indicum* toward the food-derived mutagen, Trp-P-1, was determined by the Ames test. The significant activity was detected in a fraction eluted with 70-80% methanol. Further purification yielded baicalein as the antimutagenic principal (Nakahara et al., 2001).

Anticancer Activity:

A study revealed that eight species of plants used in Bangladeshi folk medicine exhibited some cytotoxic activities in the brine shrimp lethality assay, sea urchin eggs assay, hemolysis assay and MTT assay using tumor cell lines. The extract of *O. indicum* showed the highest toxicity on all tumor cell lines tested, with an IC_{50} of 19.6 mg/mL for CEM, 14.2 mg/mL for HL-60, 17.2 mg/mL for B-16 and 32.5 mg/mL for HCT-8 (Costa-Lotufo et al., 2005).

Antiproliferative Activity:

A study was conducted to analyze the antiproliferative activity of several Bangladeshi medicinal plant extracts on different human cell lines including erythroleukemic K562 cells, B lymphoid Raji and T lymphoid Jurkat human tumor cell lines. The data obtained indicate that the ethanolic extract of the stem bark of *O. indicum* showed an antiproliferative activity on all analyzed human tumor cell lines: erythroleukemic K562 cells (IC_{50}=3.77±0.32 mg/mL), B lymphoid Raji (IC_{50}=23.20±9.6 mg/mL) and T lymphoid Jurkat (IC_{50}=4.11±0.1 mg/mL).

The same plant extracts were screened for their activity in inhibiting the interactions between nuclear factors and double stranded target oligonucleotides mimicking the transcription factors such as nuclear factor-kappa B (NF-κB), activator protein (AP-1), signal transducer and activator of transcription (STATs), cAMP response element binding protein (CREB) and GATA-1 factors. The results showed that high concentration of *O. indicum* extract was unable to inhibit almost all TFs/DNA interactions, while it is active on the other AP-1/DNA interactions only when added at 50 mg/mL (Lampronti et al., 2008).

Antispasmodic Activity:

An ethanol-water (1:1) extract of fruit had an antispasmodic activity on the isolated guinea pig ileum but had no effect on rat uterine smooth muscle (Dhar et al., 1968).

Gastroprotective Activities

The gastroprotective activities were investigated for crude extracts of stem bark of *O. indicum* and the isolates against various gastric ulceration models in Wistar rats. At two random dosages of 100 mg and 250 mg/kg body weights, hexane and acetone extracts displayed mild to moderate gastroprotective activity. Acetone extract displayed better activity than hexane extract. (Hari Babu et al., 2010)

Cytotoxic Activity:

An ethanol-water (1:1) extract of root and fruit were not cytotoxic against 9KB cell (Dhar et al., 1968).

Toxicity Studies:

An ethanol-water (1:1) extract of bark and root had a maximum tolerated dose at 1.0 g/kg i.p in mice (Dhar et al., 1968). Three fraction of 70% ethanol extract of bark were tested for acute and subchronic toxicities in mice. The results revealed that when orally administered at doses up to 800 mg/kg, every fraction had no acute or subchronic toxicity. But intraperitoneal injection of either the first or the second fraction caused mortality (Glinsukon, 1987).

Dimocarpus longan **Lour.**


Figure 5 *Dimocarpus longan* Lour. fruit and *Dimocarpus longan* root

Family Sapindaceae

Synonyms

- ssp. *longan* var. *longan*: *Dimocarpus longan* Lour. (1790), *Euphoria longana* Lamk (1792) nom. illeg., *Nephelium longana* Cambess. (1829).

- ssp. *longan* var. *longepetiolulatus* Leenh.: *Euphoria morigera* Gagnep. (1950) nom. inval.

- ssp. *longan* var. *obtusus* (Pierre) Leenh.: *Euphoria scandens* Winit & Kerr.

- ssp. *malesianus* Leenh. Var. *malesianus*: *Nephelium malaiense* Griff. (1854), *Euphoria cinerea* Radlk. (1878) nom. illeg., *Euphoria malaiensis* Radlk. (1879) nom. illeg., *Euphoria gracilis* Radlk. (1913) nom. illeg.

- ssp. *malesianus* Leenh. var. *echinatus* Leenh.

Vernacular names

- ssp. *longan* var. *longan*: longan (En). Longanier, oeil de dragon (Fr). Indonesia, Malaysia: lengkeng. Burma: kyet mouk. Cambodia: mien. Laos: lam nhai, nam nhai. Thailand: lamyai pa. Vietnam: nhan.

- ssp. *longan* var. *obtusus*: Thailand: lamyai khruer, lamyai tao.

- ssp. *malesianus* var. *malesianus*: Malaysia: mata kucing (Peninsular Malaysia and Sabah), isau, sau, kakus (Sarawak). Indonesia: buku, ihau (Kalimantan), medaru (Sumatra).

Distribution

Ssp. *longan* var. *longan*: Whereas some authors limit the area of origin to the mountain chain from Burma through southern China, others extend it to south-west India and Sri Lanka, including the lowlands. The crop is mainly grown in south China, Taiwan and north Thailand with small acreages elsewhere in Indo-China as well as Queensland (Australia) and Florida (United States) and scattered trees at higher elevations in South-East Asia.

Ssp. *longan* var. *longepetiolulatus*: southern Vietnam.

Ssp. *longan* var. *obtusus*: Indo-China, cultivated in Thailand.

Ssp. *malesianus* var. *malesianus*: all over Indo-China and Malesia, greatest variation found in Borneo.

Ssp. *malesianus* var. *echinatus*: Borneo and the Philippines.

Observation

Tree, up to 40 m tall and 1 m trunk diameter, sometimes buttressed, exceptionally a scandent shrub; branches terete with 5 faint grooves, sometimes warty lenticellate, rather densely ferruginous tomentose.

Leaves 2-4(-6)-jugate, axial parts mostly densely hairy; petiole 1-20 cm, petiolules 0.5-35 mm long; leaflets elliptical, 3-45 cm x 1.5-20 cm, 1-5 times longer than wide, chartaceous to coriaceous, above often tomentose in basal part of midrib, beneath thinly tufted-tomentose mainly on midrib and nerves. Inflorescences usually terminal, 8-40 cm long, densely tufted-tomentose; cymules (1-)3-5-flowered; pedicels 1-4 mm; bracts patent, 1.5-5 mm long; flowers yellow-brown; calyx lobes 2-5 mm x 1-3 mm; petals 5, 1.5-6 mm x 0.6-2 mm, densely woolly to glabrous; stamens (6-)8(-10), filament 1-6 mm.

Fruit drupaceous, 1-3 cm in diameter, lobe(s) broad-ellipsoid to globular, smooth to warty or sometimes up to 1 cm aculeate, sometimes granular, glabrescent, yellow-brown. Seed globular with shining blackish-brown testa; seed enveloped by a thin fleshy, translucent white arilloid.

Uses

Longans as well as the minor fruits of the species are mainly eaten fresh. There are substantial canning industries for longan in Thailand, China and Taiwan. Large fruits

are used, preferably those with small seeds. Fruit can be canned in its own juice with little or no sugar, due to the high level of soluble solids. Canned longans retain their individual flavor better than do rambutan or lychee. Longans can be preserved dry, either intact or after removal of the pericarp. The dried flesh is black, leathery and smoky in flavour and is used mainly to prepare a refreshing drink. A liqueur is made by macerating the longan flesh in alcohol.

The seeds are used as a shampoo, like soap berries (*Sapindus saponaria* L.), because of their saponin content. Both the seed and the fruit flesh of longan have several medicinal uses.

The leaves, which contain quercetin and quercitrin, and flowers are sold in Chinese herb markets. The red, hard longan timber and the fairly hard, light brown to yellow 'mata kucing' timber are useful, but rarely available. In eastern Thailand ssp. *longan* var. *obtusus* is grown as an ornamental climber (Choo and Ketsa, 1991).

Phytochemicals

Significant amounts of phenolics, fatty acids and proteins exist in longan fruit. Phenolic compounds including gallic acid, corilagin, ellagic acid and their conjugates, (-)-epicatechin, 4-O-methylgallic acid, flavone glycosides, glycosides of quercetin and kaempferol from longan fruit pericarp (Jaitrong et al., 2006; Rangkadilok et al., 2005; Shi et al., 2008; Sun et al., 2007), and ethylgallate1-β-O-galloyl-D-glucopyranose, methyl brevifolin carboxylate, grevifolin and 4-O-α-L-rhamnopyranosyl-ellagicacid, gallic acid, corilagin and ellagic acid from longan seed (Zheng et al., 2009) have been identified.

Longan aril contains lysophosphatidyl choline, phosphatidyl choline, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanol amine, phosphatidate and phosphatidic acid glycerol (Sheng and Wang, 2010). Such phospholipids can be metabolized by a variety of membrane lipid-related enzymes and may purportedly enhance immune function in human consumers. In addition, longan pericarp contains significant amounts of polysaccharides (Jiang et al., 2009a). The polysaccharides are mainly composed of L-arabinofuranose (32.8%), D-glucopyranose (17.6%), D-galactopyranose (33.7%) and D-galacturonicacid (15.9%) (Yang et al., 2009).

Pharmacological Activities

Antioxidant Activity

There is some direct evidence for the antioxidant potential with phenolic compounds in longan fruit (de Assis et al., 2009; Wen et al., 2010). Guo et al., (2003) compared the antioxidant activities of peel, pulp and seed fractions of longan fruit using the FRAP assay, while Prasad et al.(2010) found that polyphenol-rich longan extract can strongly inhibit linoleic acid oxidation and exhibit a dose-dependent free-radical scavenging activity against DPPH radicals, superoxide anion and hydroxyl radicals. The different total antioxidant capacities were reported in longan arils of various cultivars. Among these 16 cultivars, 'Kusan' exhibits the lowest FRAP value of 4.19 mmol/L (Yamaguchi et al., 2000). Furthermore, longan polysaccharides showed a good antioxidant activity *in vitro* (Jiang et al., 2009b). However, methylation of polysaccharides from longan pericarp reduced radical scavenging activity (Yang et al., 2010). In addition, phenolics from longan pericarp have also found to have excellent reducing power (Duan et al., 2007).

Anti-tyrosinase activity

Tyrosinase is a multifunctional enzyme that catalyzes both the hydroxylation of monophenols such as tyrosine to o-diphenols and the oxidation of o-diphenols to o-quinones. Meanwhile, the enzyme is widely distributed in organisms and plays an important role in melanin production. Tyrosinase inhibitors may be clinically useful for the treatment of skin cancer and some dermatological disorders associated with melanin hyper pigmentation and are important in cosmetics for whitening and depigmentation after sunburn (Shaheen et al., 2005).

Rangkadilok et al. (2007) determined standardized longan fruit extract. Furthermore, Yang et al. (2009) found that polysaccharides from longan fruit pericarp can strongly inhibit the tyrosinase activity and act as a noncompetitive inhibitor of the enzyme. The inhibition of tyrosinase activity by the major constitutes of longan polysaccharides needs to be investigated further.

Anti-glycated Activity

Glycation has been confirmed to have a significant role in diabetic complications and normal aging (Yamaguchi et al., 2000). Polysaccharides from longan pericarp

showed a good and stable anti-glycated activity (Yang, Zhao and Jiang, 2009). Ultrasonic-assisted extraction technique can increase the anti-glycated activity of polysaccharides obtained from longan pericarp. The role of longan potential polysaccharides in preventing diabetics and normal aging is worth further evaluation.

Anticancer Activity

Polyphenol-rich longan seed extract is a free-radical scavenger that possesses known pharmacological properties and is used by humans for therapeutic purposes. Prasad, et al. (2009) reported the anticancer activity of pericarp extract of longan fruit against the HepG2, A549, and SGC7901cancer cell lines. The extract from longan pericarp obtained by the high pressure-assisted extraction showed higher anticancer activity than the conventional extraction. Furthermore, polyphenol-rich longan seed extract also inhibited the proliferation of Colo320DM, SW480 and HT-29 by blocking cell cycle progression during the DNA synthesis phase and inducing apoptotic death, reduced the expression of cyclin A and cyclin D1, activated caspase3 and increased the Bax/Bcl-2 ratio (Chung et al., 2010). It was suggested that a polyphenol-rich longan extract can be employed as a potential novel treatment agent for cancer.

Other beneficial effects

Longan fruit has been used for the traditional Chinese medicine formulation to decrease the neural pain and swelling. Besides the anti-tyrosinase, antiglycated and anticancer activities, other therapeutic potential of longan fruit have also been found.

Park et al. (2010) reported that subchronic administration of the aqueous extract of longan fruit could enhance learning and memory, and its beneficial effects are mediated, in part, by BDNF expression and immature neuronal survival. Currently, the information on other therapeutic uses of longan fruit is very limited, and, thus, other beneficial effects such as antibacterial activity, anti-obesity, and antiviral properties of longan fruit could be evaluated.

Dolichandrone serrulata DC. Seem.

Figure 6 *Dolichandrone serrulata* (DC.) Seem.

Family Bignoniaceae

Synonyms *Stereospermum serrulata* DC.

Observation

Deciduous tree to 25 m. with narrow cylindrical crown & slender branches.

Bark: pale brown, smooth of slightly flaking.

Leaf: to 43 cm., once-pinnate,3-5 pairs of leaflets, 5-14x3-6 cm, elliptic with tapering tip & strongly asymmetric base, usually with scattered teeth. Young leaves slightly sticky, mature leaves smooth or with tufts of hairs in vein axils below & a few large glands on the midvein. Leaflet stalks 0.5-1.3 cm.

Flower: 12-21 cm. pure white, opening at night , in short unbranched clusters of 3-7 flowers at end of twigs, 2-3 cm. Individual flower stalks 1.8-3.8 cm. buds narrowly conical & slightly curved, 3-5 cm. Calyx 3-5 cm, pale green with many glands, deeply split on one side only, spathe-like. Corolla narrowly tubular in the bottom half, funnel shaped in the top half (both parts + same length). With spreading, wavy lobes. Stamens not projecting beyond corolla.

Fruit: up to 85x1.8 cm. pointed, spirally twisted, seeds 2.2-2.8x0.5-0.8 cm. rectangular, thin with transparent wing.

Uses

The flower of this plant has a bitter taste and has been used as a vegetable.

The bark is used in Thai traditional medicine as an antifever and anti-inflammatory agent (Sinaphet et al., 2006).

Phytochemicals

A phenolic triglycoside, dolichandroside, was isolated from the branches of *Dolichandrone serrulata* together with decaffeoyl-verbascoside, verbascoside, isoverbascoside, markhamioside A, 2-O-apiosylver-bascoside, luteoside bandixoside (Sinaphet et al., 2006).

Walsura trichostemon Miq.

Figure 7 *Walsura trichostemon* Miq.

Family Meliaceae

Observation

Botany evergreen of briefly deciduous trees, very rarely with latex of sap height of 4-15 meters, medium brown bark.

Leaves odd-pinnate, stalks swollen & jointed. Alternate, spirally arranged, leaflets usually opposite, no stipules.

Flowers mostly white or yellow, regular, bisexual, in branched clusters at upper leaf axils, 4-5 free spreading petals, stamens longer than petals, style short, disc ring-like.

Fruits fleshy or leathery, not splitting, 1-2 seeds with aril.

Distribution

Walsura trichostemon Miq is a plant family Meliaceae that has been found in evergreen forest drought throughout Southeast Asia such as Myanmar, Cambodia. Thailand found in North, Northeast and southeastern, which know the local name of Musk Mallow tree (Polyium, Ta-Ngam and Thongnoi, 2009).

Uses

Apart from *W. trichostemon* are using in Thai traditional medicine, as an tendon disabilities, staunch, wash the wound, hemorrhoids and eating to reduce irritation (Polyium et al., 2009) and are used for paresis (Chuakul and Boonpeng, 2004).

Pharmacological Activities

Antimycobacterial and Cytotoxic activity

Antibacterial screening showed the crude methanol extract had a good activity against isolated bacterial strains (MIC between 62.5 and 125 µg/ml). *S. milleri*, *S. aureus* MRSA 2036 21083, *S. aureus* ATCC 25923 and *S. Mutans* ATCC 27175 were the most sensitive bacteria (MIC = 62.5 µg/ml). *S. pneumonia*, *S. aureus* (MRSA), *B. subtilis* ATCC 26633 and *B. pertussis* showed MIC value of 125 µg/ml (Chotsang, Aroonrerk, and Charoenying, 2006).

Extractions of bioactive constituent from the stem bark of *W. trichostemon* with a polarity sequential extraction and maceration technique with hexane, ethyl acetate and methanol, respectively. Crude extracts were tested for their antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra strain (Anti-TB) using the green fluorescent protein microplate assay (GFPMA) and cytotoxic activity against human mouth carcinoma (KB), human small cell lung cancer (NCI-H187) and breast cancer (MCF-7) cancer cell lines, tested using the resazurin microplate assay (REMA). Crude extract showed inhibitory effect against *M. tuberculosis* with MIC_{50} of 50 g/ml and showed inhibitory effect against KB, NCI-H187 and MCF-7 cancer cell lines with IC_{50} values of 1.35- 36.38 g/ml (Polyium et al., 2009).

The biological activity of the leaf of *W. trichostemon* with a polarity sequential extraction and maceration technique with hexane, ethyl acetate and methanol, respectively. Crude extracts were tested for their antimycobacterial activity against *M. tuberculosis* H37Ra strain (Anti-TB) using the green fuorescent protein microplate assay (GFPMA) and anti-proliferative assay for cancer cell lines against human mouth carcinoma (KB), human small cell lung cancer (NCI-H187) and breast cancer (MCF-7) cancer cell lines, tested using the resazurin microplate assay (REMA). Crude ethyl acetate extract showed inhibitory effect against *M. tuberculosis* with MIC of 50 µg/mL. Crude hexane, ethyl acetate and methanol extract showed inhibitory effect against KB with IC_{50} values of 10.42- 40.14 µg/ml., NCI-H187 with IC_{50} values of 17.02-25.76 µg/ml and MCF-7 with IC_{50} values of 26.07-36.37 µg/ml. The results showed that the extracts from the leaf of *W. trichostemon* have a significant antimycobacterial and cytotoxic activity (Polyium and Malaphan, 2010).

CHAPTER III

MATERIALS AND METHODS

ANIMALS

Male Wistar rats (5 weeks of age; weighing 140-180 g) from National Laboratory Animal Center, Mahidol University, Salaya, Nakornprathom were served as experimental subjects. They were housed in the animal facility of the Faculty of Pharmaceutical Sciences, Chulalongkorn University under the standard condition of temperature (25 $\pm$ 2^{o}C), 50-60% of humidity, 12 hr/12 hr light/dark cycles and had accessed to the standard pellet diet (Perfect Companion Group Company Limited, Thailand) and tap water *ad libitum*. The animals were allowed to acclimate to the facility for 3-5 days before starting the experiments. At the end of each experiment, the animals were sacrificed with carbon dioxide. The number of animals used in each treatment was six per group. The study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University (Appendix A).

DRUGS AND CHEMICALS

The following drugs and chemical were used: 0.9% sodium chloride solution, 2% Tween 80 , acetylsalicylic acid (aspirin) (300 mg/kg, Sigma Chemical Co., USA), lipopolysaccharide (LPS) from *E. coli* (50 μg/kg, Sigma Chemical Co., USA), extracts of individual components in Ben-Cha-Moon-Yai remedy: *Aegle marmelos* root extract (AM; 25-400 mg/kg), *Oroxylum indicum* root extract (OI; 25-400 mg/kg), *Dimocarpus longan* root extract (DL; 25- 400 mg/kg), *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg) and *Walsura trichostemon* root extract (WT; 25-400 mg/kg); and the whole extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). See Appendix B for preparation and identification.

LPS was dissolved in 0.9% sodium chloride solution. Extracts of five components of Ben-Cha-Moon-Yai remedy, Ben-Cha-Moon-Yai remedy, and aspirin were suspended

in 2% Tween 80. Aspirin was used as a standard antipyretic drug. The control animals were given with equivalent volume of vehicle in the same route.

EXPERIMENTAL METHODS

Lipopolysaccharide-induced fever in rats

The method of Santos and Rao in 1998 was modified and used for the assessment of the antipyretic activity of the whole extract of Ben-Cha-Moon-Yai remedy (BMY) and various doses of extracts of individual components in Ben-Cha-Moon-Yai remedy (AM, OI, DL, DS, and WT). The animals were fasted overnight before the experiments. Each animal was kept in a restrainer for 1 hr to acclimatize to its new environment. Fever was induced with 50 μg/kg of LPS injected intramuscularly into the thigh of the rat. The animals were pretreated orally with 2% Tween 80 solution (10 ml/kg), acetylsalicylic acid (ASA; 300 mg/kg), various doses of AM, OI, DL, DS, and WT (25, 50, 100, 200 and 400 mg/kg) or BMY (125, 250 and 500 mg/kg) 1 hr before injection of LPS. Normal rats were received 2% Tween 80 solution (10 ml/kg) orally 1 hr before 0.9% normal saline solution (NSS) injection. Rectal temperature was measured 1 hr before the pretreatment of animals and at 1 hr intervals for 7 hr after the administration of the bacterial endotoxin (LPS) with a lubricated digital thermometer (YSI Precision™ model 4000A, USA) inserted 3-4 cm deep into the rectum of the rats. The rectal temperature of normal rats was also measured at 1 hr intervals for 7 hr. The control experiment involved animals treated with 2% Tween 80 plus LPS. All experiments were carried out between 08.00 h and 18.00 h in a quiet laboratory with an ambient temperature of 25 ± 2°C.

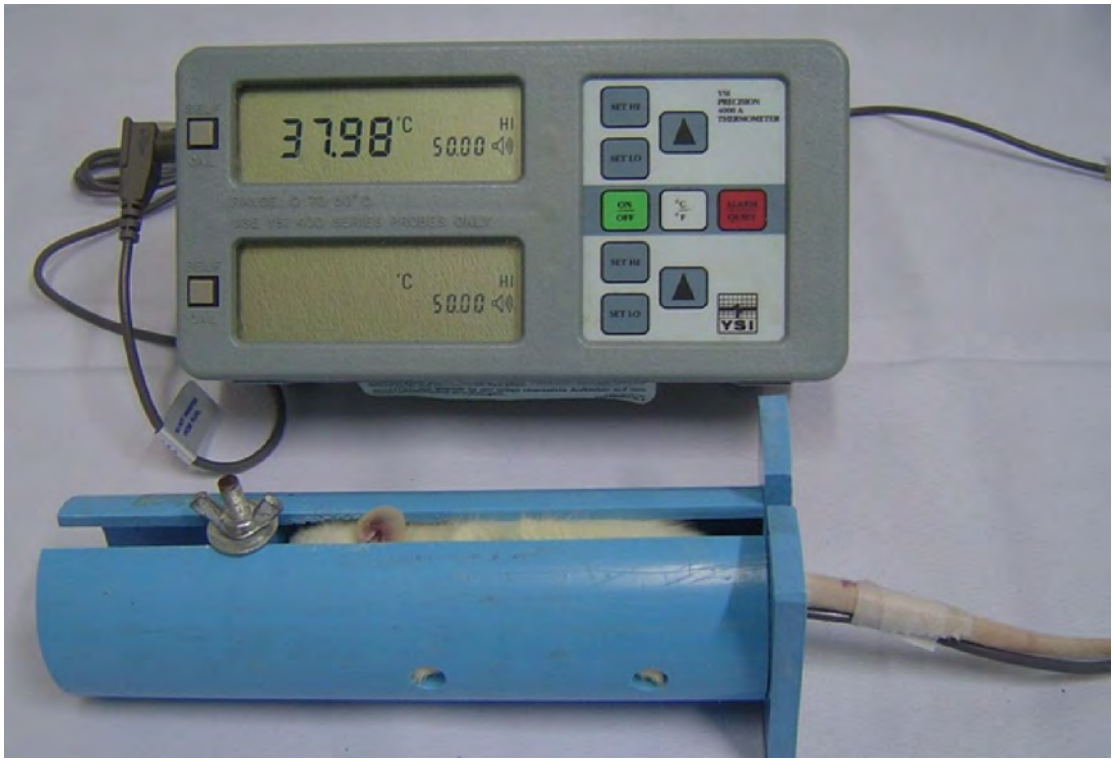

Figure 8 Digital Thermometer (YSI Precision™ 4000A)

DATA TREATMENT AND STATISTICAL ANALYSE

Statistical analyses were performed on the dose-response and were reported for the group as mean ± standard error of the mean. The areas under the temperature-time response curves (AUC) for each rat were determined by Trapezoidal integration after subtracting the respective basal levels from each subsequent value, and were used for tests of statistical significance of treatments. The AUC differences (Δ AUC) were determined by subtracting AUC of control LPS from AUC of each treatment. For statistical significance the data were analyzed by one-way analysis of variance followed by Fisher LSD method (SPSS version 13.0 for windows).The minimum level of statistical significance was set at $p<0.05$.

CHAPTER IV

RESULTS

I. Effects of an extract from Ben-Cha-Moon-Yai remedy on LPS-induce fever

Lipopolysaccharide (LPS; 50 µg/kg) injected intramuscularly significantly (p<0.001) produced a time-dependent increase in rectal temperature in vehicle pretreated rats starting from 1 hr and this effect was maintained for 7 hr after LPS injection. The maximum increase in rectal temperature was reached at 2 hr (0.89°C) giving a maximum observed mean rectal temperature of 38.69 ± 0.14°C after which there was a decrease (Figure 9). At the same time, the mean rectal temperature of normothermic rats was 37.80 ± 0.15°C. Thus, LPS significantly (p<0.001) increased the rectal temperature (Figure 9).

Acetylsalicylic acid (ASA; 300 mg/kg) significantly (p<0.05) reduced the increased rectal temperature produced by LPS over a period of 7 hr with a maximum reduction at 2 hr. The mean rectal temperature produced by LPS in the presence of ASA was reduced to 37.68 ± 0.23°C (Figure 9).

BMY at the dose of 125 mg/kg significantly (p<0.01) attenuated the increase in rectal temperature produced by LPS starting at 2 hr and the effect was maintained for the full 7 hr with a maximum reduction at 3 hr after LPS injection. BMY at the dose of 250 mg/kg significantly reduced LPS-induced increase in rectal temperature at 2 and 3 hr (p<0.05 and p<0.01, respectively) with a maximum reduction at 3 hr after LPS injection. BMY at the dose of 500 mg/kg significantly (p<0.01) attenuated the increase in rectal temperature produced by LPS starting at 1 hr and the effect was maintained for the full 7 hr with a maximum reduction at 3 hr after LPS injection (Figure 10).

LPS significantly (p<0.001) increased the area under the temperature-time response (AUC) compared to normal rats. ASA significantly (p<0.001) decreased the AUC compared to control LPS. All doses of BMY (125, 250, and 500 mg/kg) significantly decreased the AUCs compared to control LPS (p<0.001, p<0.05 and p<0.001, respectively). BMY at the dose of 500 mg/kg seemed to have the highest antipyretic efficacy (Figure 11).

II. Effects of an extracts from individual components in Ben-Cha-Moon-Yai remedy on LPS-induce fever

1. The root extract of *Aegle marmelos* (AM)

AM at the doses of 25 and 50 mg/kg significantly ($p<0.05$) reduced LPS-induced increase in rectal temperature at 2 and 3 hr after LPS injection and both doses showed a maximum reduction at 3 hr. AM at the dose of 200 mg/kg significantly ($p<0.05$) reduced LPS-induced increase in rectal temperature at 3 hr after LPS injection. AM at the dose of 400 mg/kg significantly ($p<0.01$) reduced the increased rectal temperature produced by LPS over a period of 2-7 hr with a maximum reduction at 3 hr (Figure 12). LPS significantly ($p<0.01$) increased the area under the temperature-time response (AUC) compared to normal rats. AM only at the dose of 400 mg/kg significantly ($p<0.01$) decreased the AUC compared to control LPS (Figure 13).

2. The root extract of *Oroxylum indicum* (OI)

OI at the dose of 25 mg/kg significantly ($p<0.05$) reduced LPS-induced increase in rectal temperature at 2 and 3 hr with a maximum reduction at 2 hr. OI at the dose of 50 mg/kg significantly ($p<0.05$) reduced LPS induced increase in rectal temperature at 3 hr. OI at the dose of 100 mg/kg significantly reduced LPS-induced increase in rectal temperature at 5, 6 and 7 hr ($p<0.05$, $p<0.05$ and $p<0.01$) with a maximum reduction at 7 hr. OI at the dose of 200 mg/kg significantly reduced LPS induced increase in rectal temperature at 2 and 3 hr after LPS injection ($p<0.01$ and $p<0.05$) with a maximum reduction at 2 hr. OI at the dose of 400 mg/kg significantly reduced LPS induced increase in rectal temperature at 2, 3, 5, 6, and 7 hr after LPS injection ($p<0.01$, $p<0.01$, $p<0.05$, $p<0.01$ and $p<0.01$, respectively) with a maximum reduction at 3 hr (Figure 14). LPS significantly ($p<0.001$) increased the AUC compared to normal rats. OI at the doses of 25, 100 and 400 mg/kg significantly ($p<0.01$) decreased the AUC compared to control LPS. OI at the dose of 400 mg/kg seemed to have the highest antipyretic efficacy (Figure 15).

3. The root extract of *Dolichandrone serrulata* (DS)

All doses of DS could not reduce LPS-induced increase in rectal temperature (Figure 16). LPS did not increase the AUC compared to normal rats. All doses of DS did not show antipyretic effect (Figure 17).

4. The root extract of *Dimocarpus longan (*DL)

DL at the doses of 50,100 and 200 mg/kg significantly (p<0.05) reduced LPS-induced increase in rectal temperature over a period of 2-3 hr with a maximum reduction at 2, 2 and 2 hr, respectively (Figure 18) . LPS significantly (p<0.05) increased the AUC compared to normal rats. All doses of DL did not show antipyretic effect (Figure 19).

5. The root extract of *Walsura trichostemon* (WT)

WT at the dose of 25 mg/kg significantly (p<0.05) reduced LPS-induced increase in rectal temperature at 4-7 hr after LPS injection with a maximum reduction at 7 hr. WT at the dose of 50 mg/kg significantly (p<0.05) reduced LPS-induced increase in rectal temperature at 4 and 7 hr. WT at the dose of 100 mg/kg significantly (p<0.05) reduced LPS-induced increase in rectal temperature at 2, 3, 4, 6 and 7 hr with a maximum reduction at 4 hr. WT at the dose of 200 mg/kg significantly (p<0.05) reduced LPS-induced increase in rectal temperature at 4-7 hr with a maximum reduction at 5 hr (Figure 20). LPS significantly (p<0.01) increased the AUC compared to normal rats. All doses of WT except the highest dose decreased the AUC compared to control LPS (Figure 21).

III. Effects of the extracts of individual components at a dose equal to the part in Ben-Cha-Moon-Yai remedy on LPS-induced fever

The area under the temperature-time curves (AUC) differences between the whole extract of Ben-Cha-Moon-Yai remedy (BMY; 125 mg/kg) or each herbal root extract at a dose of 25 mg/kg (equal to the part in BMY 125 mg/kg) and LPS was compared. The herbal root extract at the dose of 25 mg/kg which had antipyretic effect were shown in the Figure. Only OI (25 mg/kg) and WT (25 mg/kg) showed antipyretic efficacy. BMY 125 mg/kg seemed to have the highest antipyretic efficacy (Figure 22).

The area under the temperature-time curves (AUC) differences between the whole extract of Ben-Cha-Moon-Yai remedy (BMY; 250 mg/kg) or each herbal root extract at a dose of 50 mg/kg (equal to the part in BMY 250 mg/kg) and LPS was compared. The herbal root extract at the dose of 50 mg/kg which had antipyretic effect were shown in the Figure. Only WT 50 mg/kg showed antipyretic efficacy. The antipyretic effect of WT 50 mg/kg was comparable to BMY 250 mg/kg (Figure 22).

The area under the temperature-time curves (AUC) differences between the whole extract of Ben-Cha-Moon-Yai remedy (BMY; 500 mg/kg) or each herbal root extract at a dose of 100 mg/kg (equal to the part in BMY 500 mg/kg) and LPS was compared. The herbal root extract at the dose of 100 mg/kg which had antipyretic effect were shown in the Figure. Only OI (100 mg/kg) and WT (100 mg/kg) showed antipyretic efficacy. BMY 500 mg/kg seemed to have the highest antipyretic efficacy (Figure 22).

IV. Effects of the most effective doses of the whole extract of Ben-Cha-Moon-Yai remedy and extracts of individual components in Ben-Cha-Moon-Yai remedy

The area under the temperature-time curves (AUC) differences between the most effective doses of BMY (500 mg/kg), AM (400 mg/kg), OI (400 mg/kg) and WT (100 mg/kg) and LPS was compared. The most effective doses of BMY and each herbal root extract were shown in the Figure. BMY (500 mg/kg), AM (400 mg/kg), OI (400 mg/kg), and WT (100 mg/kg) showed antipyretic efficacy in LPS-induced fever model. Most effective doses of BMY, AM, OI and WT had comparable antipyretic effect (Figure 23).

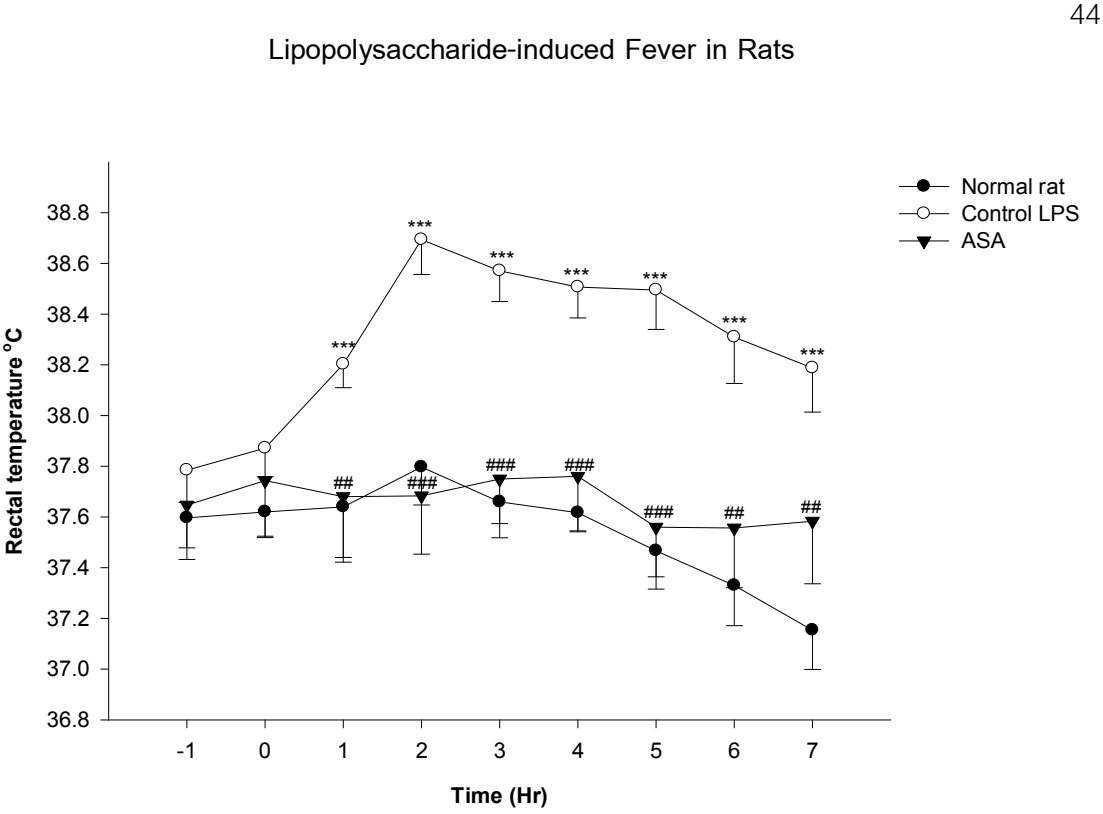

Figure 9 Changes in rectal temperature after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg). Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. Normal rats were received 0.9% NSS injection instead of LPS. N=6 for all groups. $^{***}p<0.001$ significantly different compared to normal rat values for the corresponding hour. $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ significantly different compared to control LPS values at the corresponding hour.

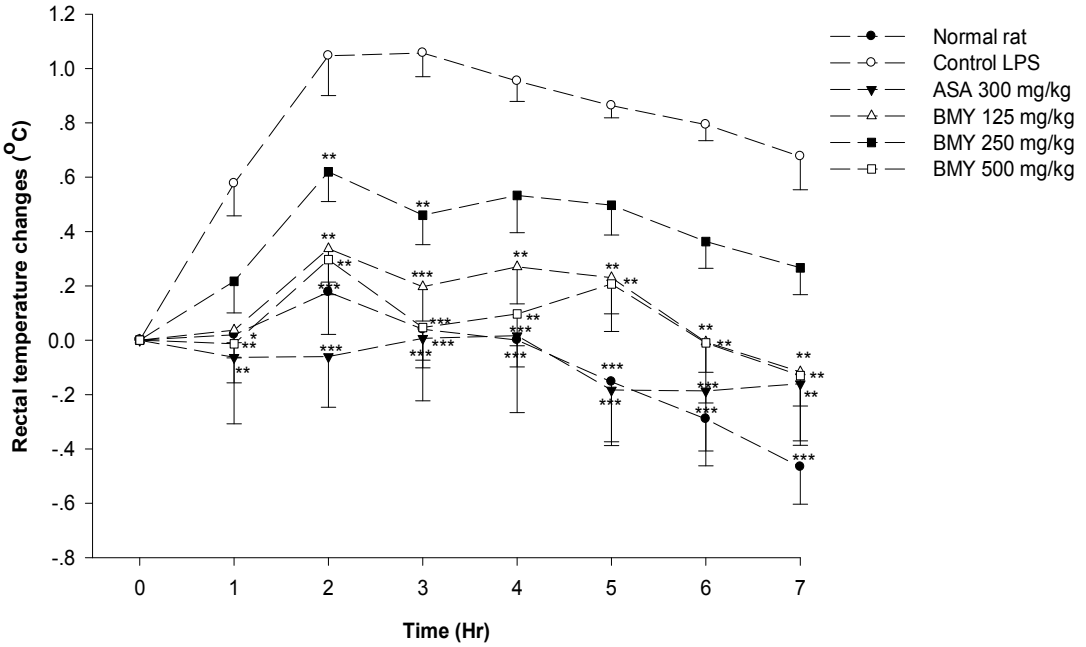

Figure 10 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. *p<0.05, **p<0.01 and ***p<0.001 significantly different compared to control LPS values at the corresponding hour.

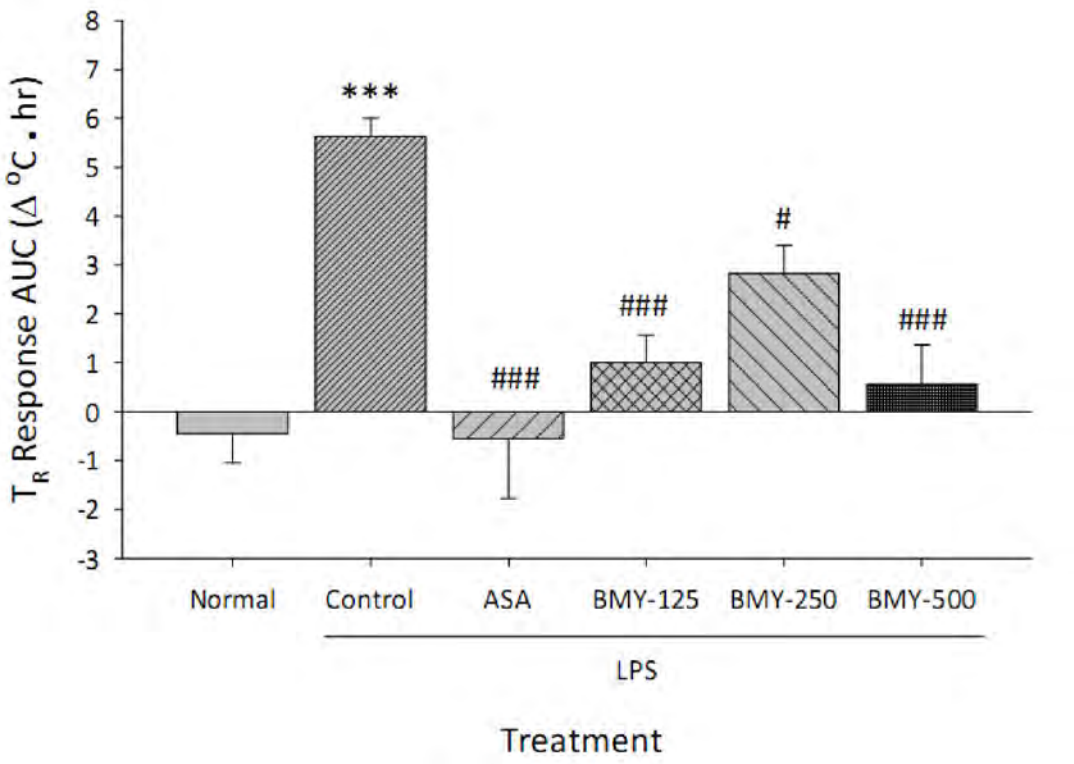

Figure 11 Area under the temperature-time response curves on lipopolysaccharide induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. ***$p<0.001$ significantly different compared to normal rat values for the corresponding hour. $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ significantly different compared to control LPS values at the corresponding hour.

The root extract of *Aegle marmelos* (AM)

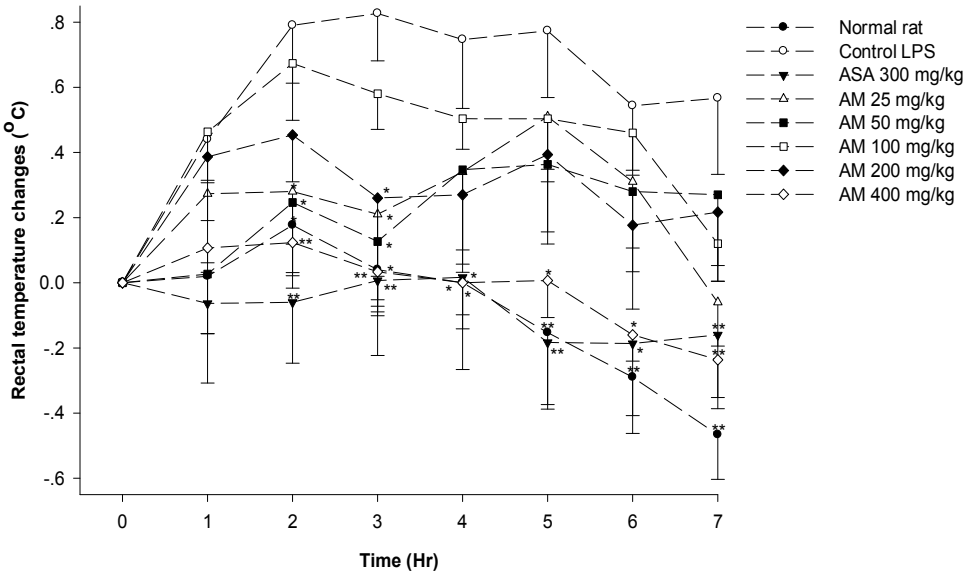

Figure 12 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Aegle marmelos* (AM; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. *$p<0.05$ and **$p<0.01$ significantly different compared to control LPS values at the corresponding hour.

The root extract of *Aegle marmelos* (AM)

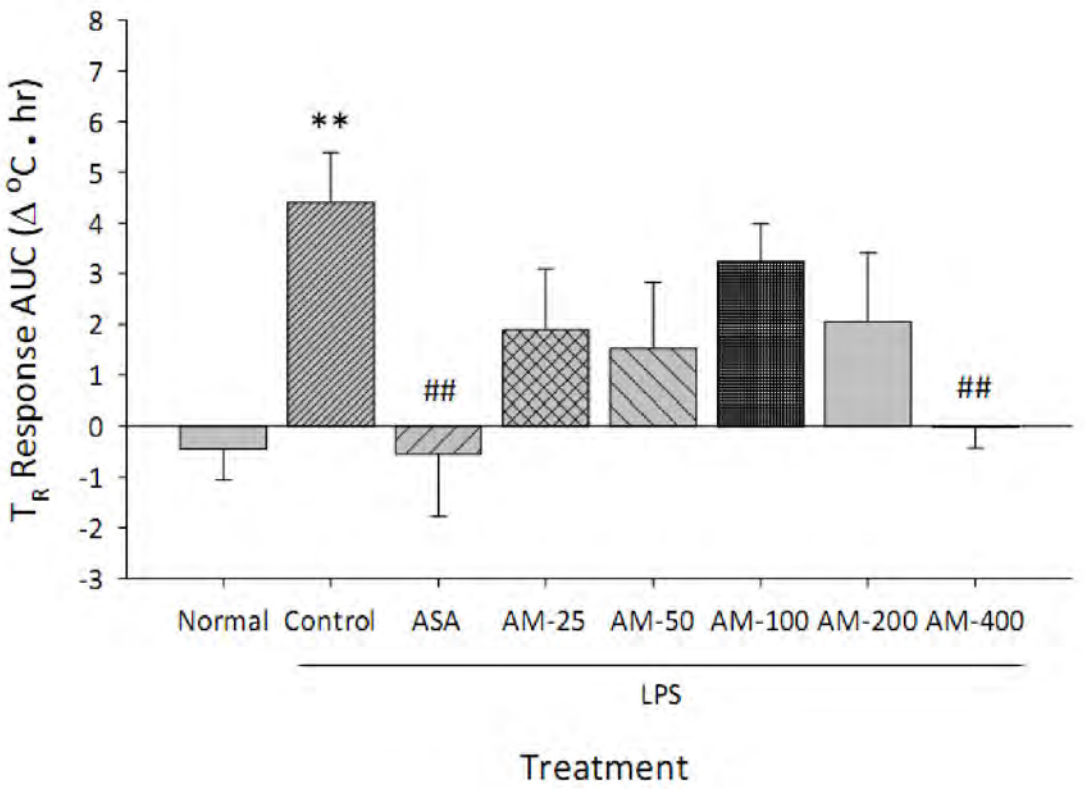

Figure 13 Area under the temperature-time response curves on lipopolysaccharide induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Aegle marmelos* (AM; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. $^{**}p<0.01$ significantly different compared to normal rat values for the corresponding hour. $^{\#\#}p<0.01$ significantly different compared to control LPS values at the corresponding hour.

The root extract of *Oroxylum indicum* (OI)

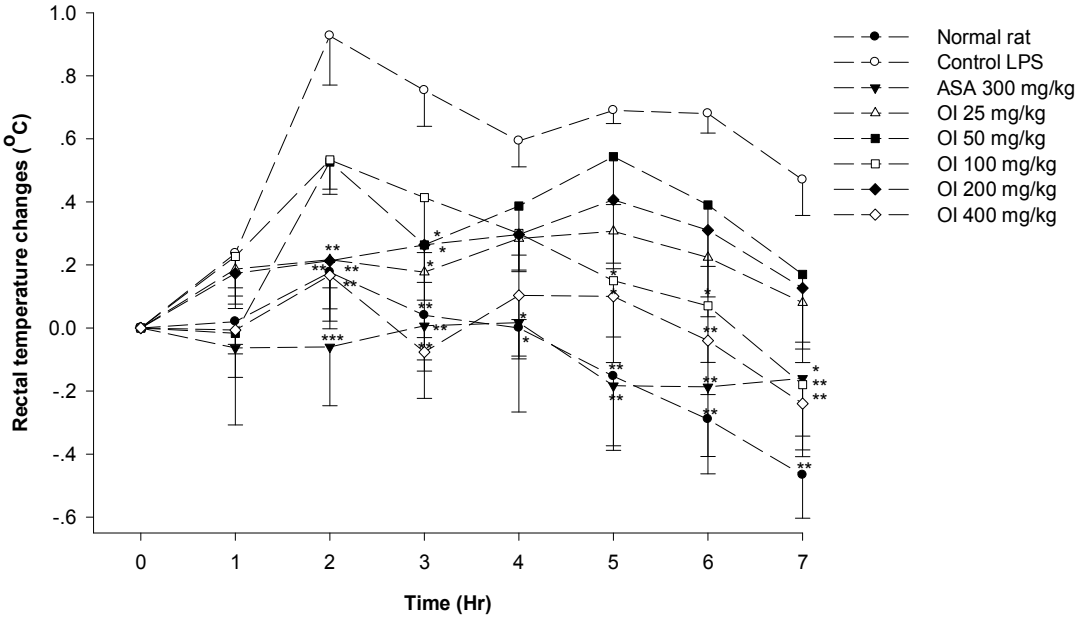

Figure 14 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Oroxylum indicum* (OI; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. *p<0.05, **p<0.01 and ***p<0.001 significantly different compared to control LPS values at the corresponding hour.

The root extract of *Oroxylum indicum* (OI)

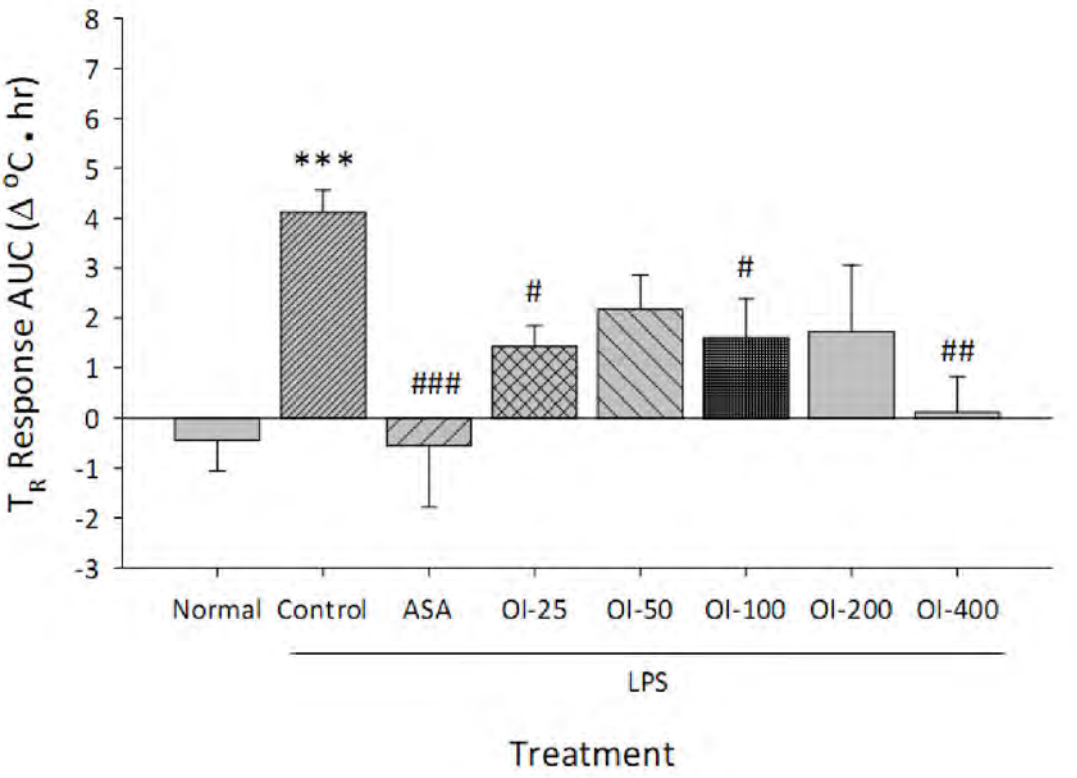

Figure 15 Area under the temperature-time response curves on lipopolysaccharide induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Oroxylum indicum* (OI; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. ***p<0.001 significantly different compared to normal rat values for the corresponding hour. $^{\#}p$<0.05, $^{\#\#}p$<0.01 and $^{\#\#\#}p$<0.001 significantly different compared to control LPS values at the corresponding hour.

The root extract of *Dolichandrone serrulata* (DS)

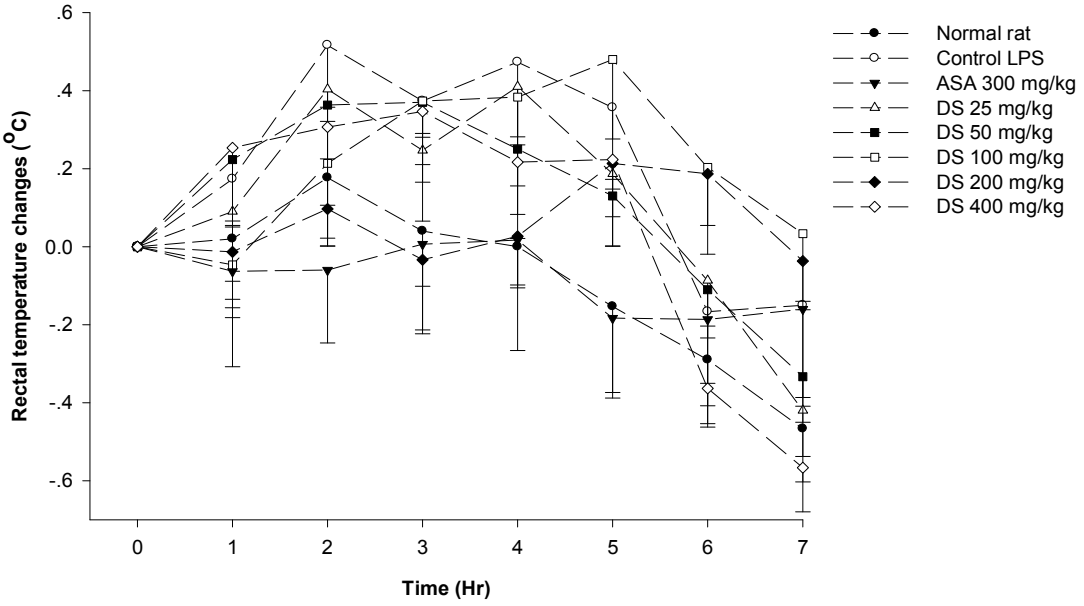

Figure 16 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Dolichandrone serrulata* (DS; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups.

The root extract of *Dolichandrone serrulata* (DS)

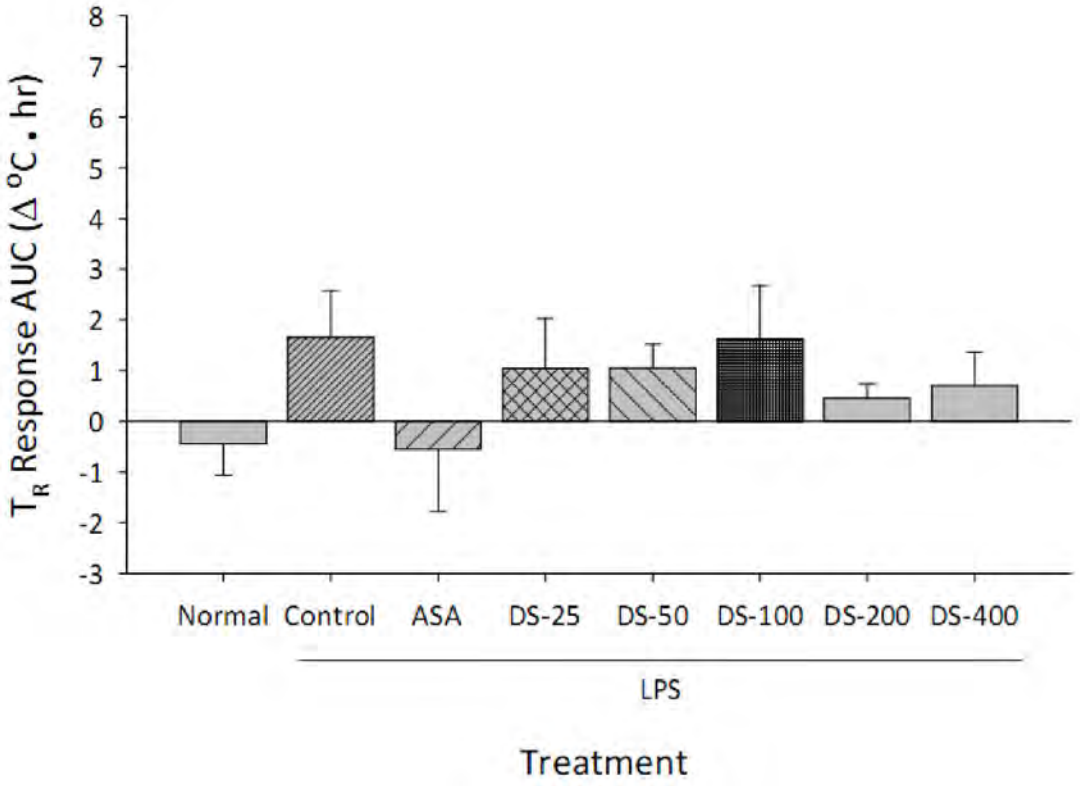

Figure 17 Area under the temperature-time response curves on lipopolysaccharide induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Dolichandrone serrulata* (DS; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups.

The root extract of *Dimocarpus longan* (DL)

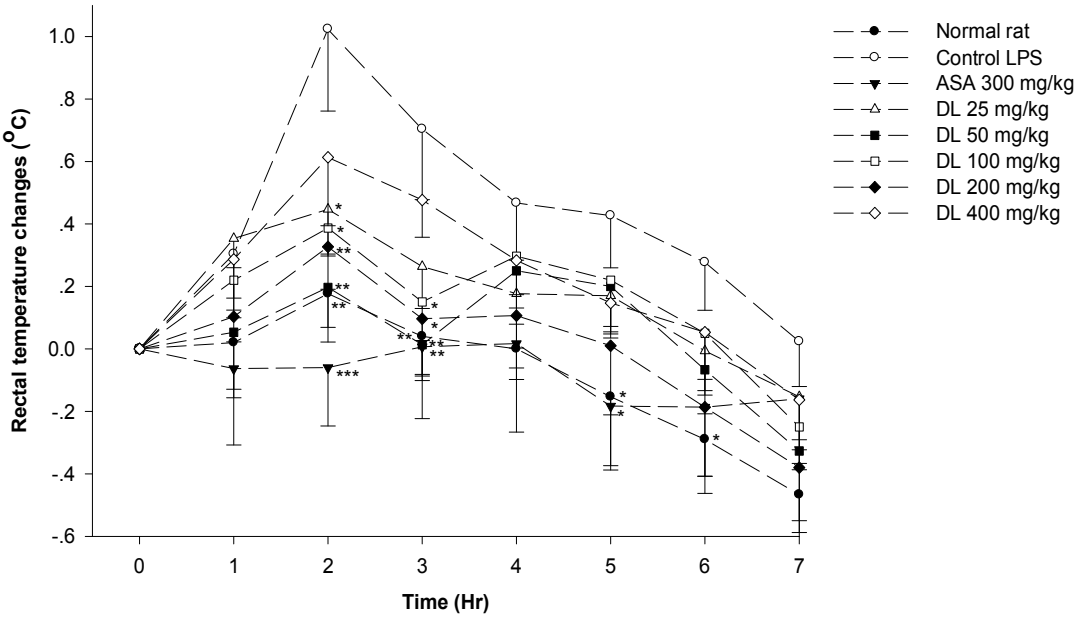

Figure 18 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract *of Dimocarpus longan* (DL; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ significantly different compared to control LPS values at the corresponding hour.

The root extract of *Dimocarpus longan* (DL)

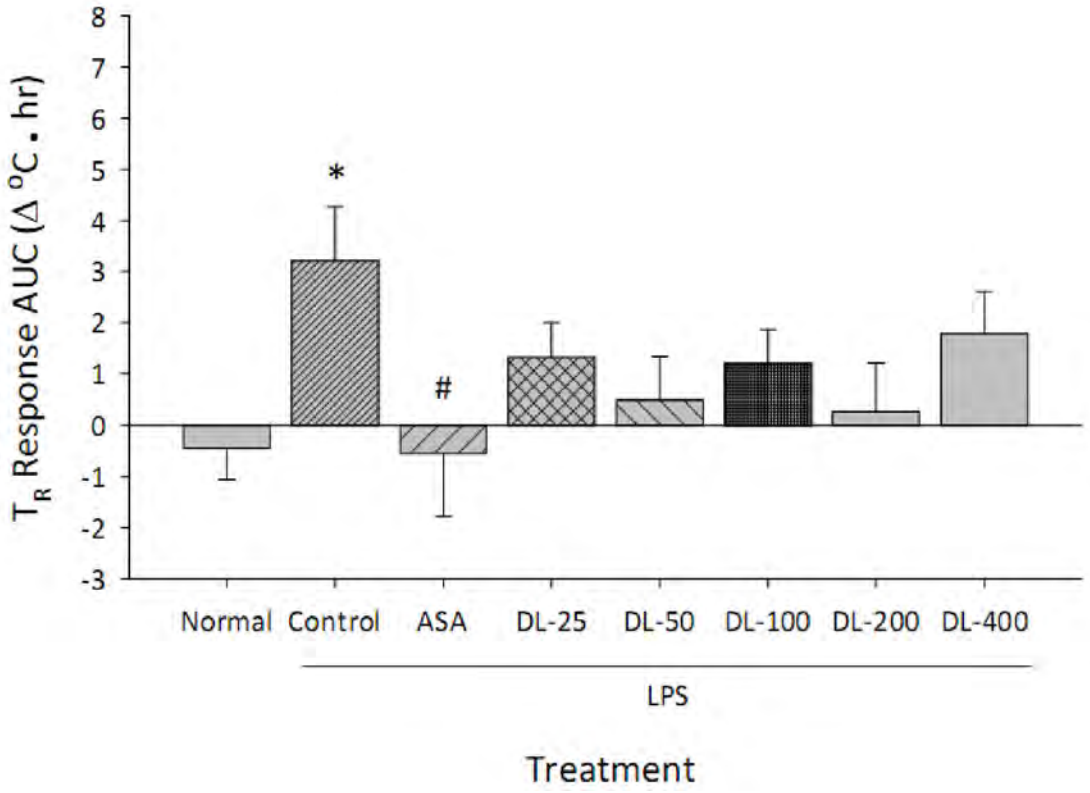

Figure 19 Area under the temperature-time response curves on lipopolysaccharide induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *of Dimocarpus longan* (DL; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. *$p<0.05$ significantly different compared to normal rat values for the corresponding hour. $^{\#}p<0.05$ significantly different compared to control LPS values at the corresponding hour.

The root extract of *Walsura trichostemon* (WT)

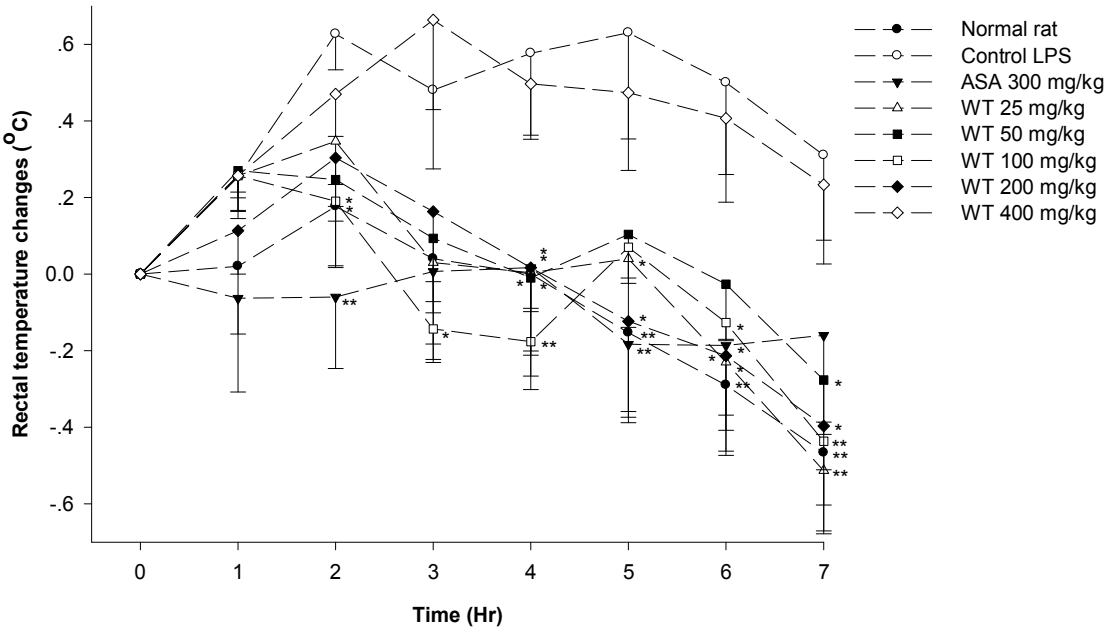

Figure 20 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Walsura trichostemon* (WT; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. *p<0.05, and **p<0.01 significantly different compared to control LPS values at the corresponding hour.

The root extract of *Walsura trichostemon* (WT)

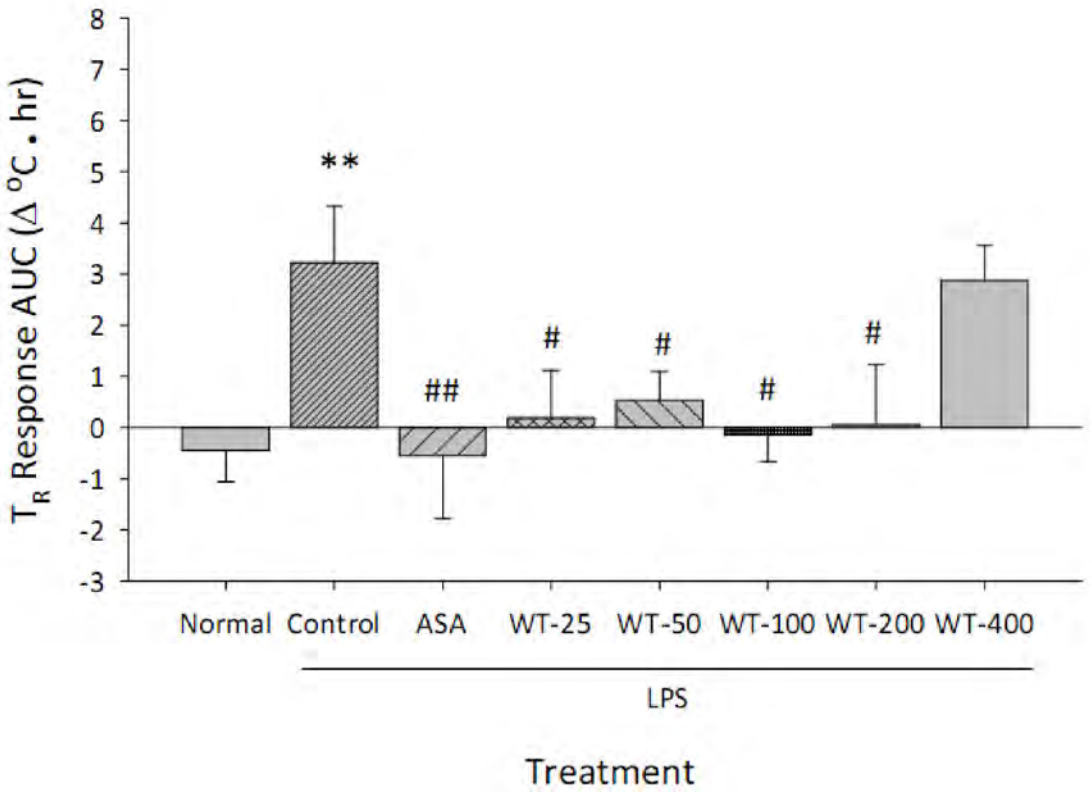

Figure 21 Area under the temperature-time response curves on lipopolysaccharide induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Walsura trichostemon* (WT; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all groups. $^{**}p<0.01$ significantly different compared to normal rat values for the corresponding hour. $^{\#}p<0.05$ and $^{\#\#}p<0.01$ significantly different compared to control LPS values at the corresponding hour.

Effects of the extracts of individual components at a dose equal to the part in Ben-Cha-Moon-Yai remedy on LPS-induced fever

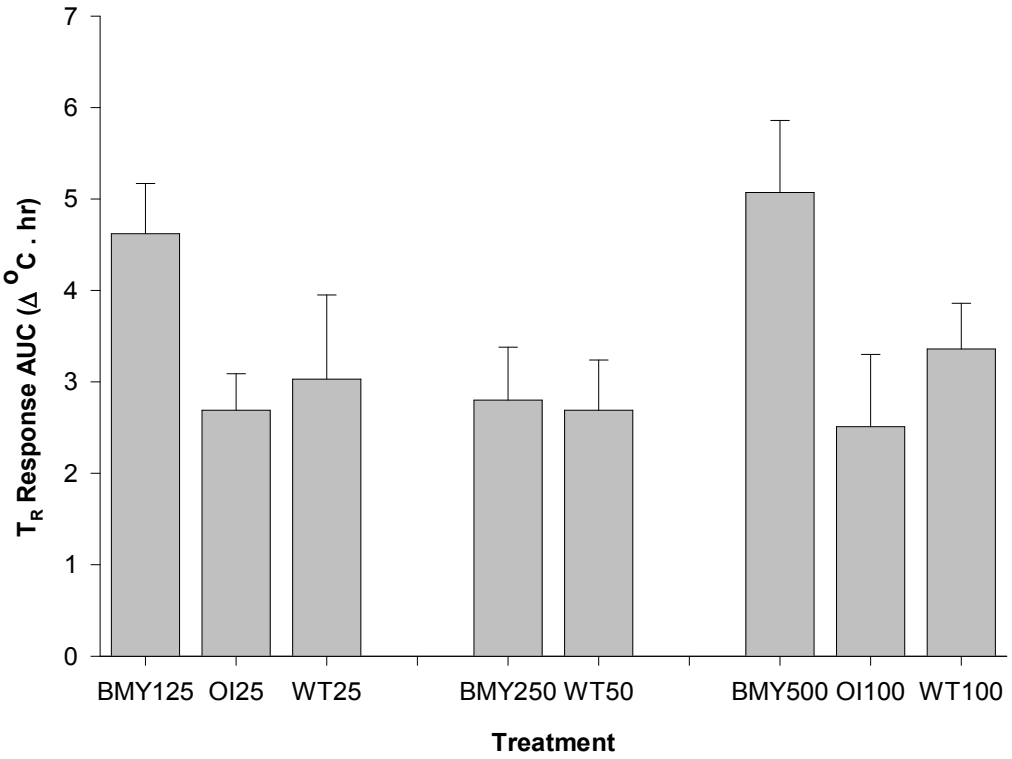

Figure 22 Differences between area under the temperature-time response curves of the whole extract of Ben-Cha-Moon-Yai remedy (BMY; 125, 250 and 500 mg/kg), the root extracts of *Oroxylum indicum* (OI; 25 and 100 mg/kg), *Walsura trichostemon* (WT; 25, 50 and 100 mg/kg) and lipopolysaccharide.

Effects of the most effective doses of the whole extract of Ben-Cha-Moon-Yai remedy and extracts of individual components in Ben-Cha-Moon-Yai remedy

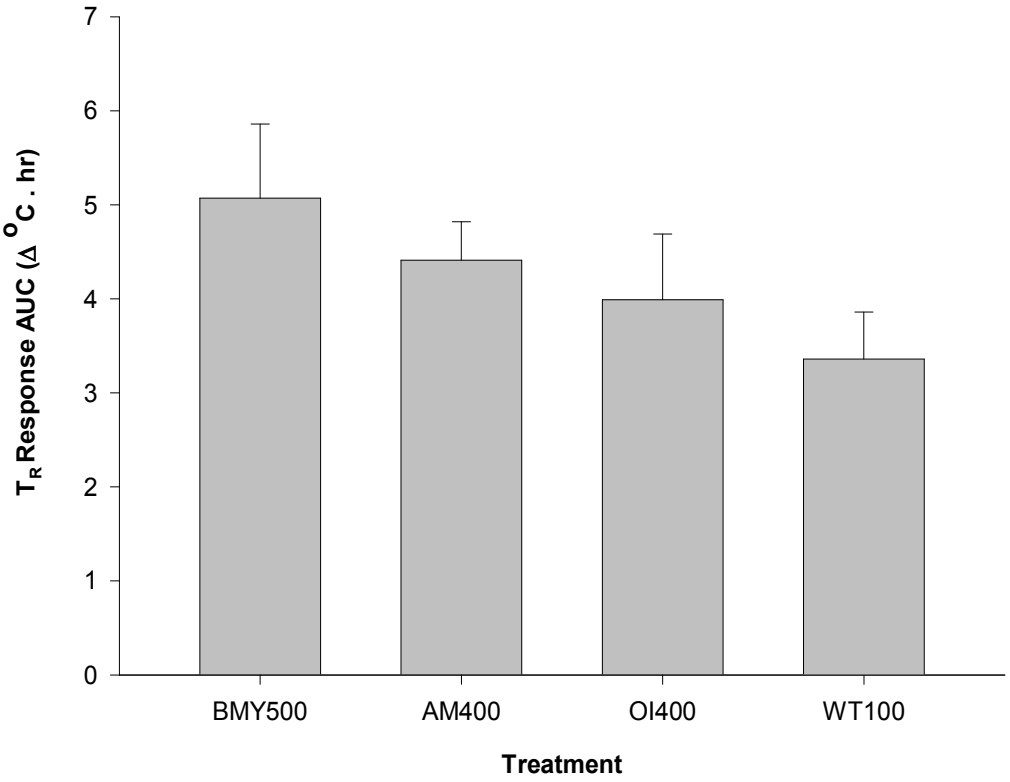

Figure 23 Differences between area under the temperature-time response curves of the whole extract of Ben-Cha-Moon-Yai remedy (BMY; 500 mg/kg), the root extracts of *Aegle marmelos* (AM; 400 mg/kg), *Oroxylum indicum* (OI; 400 mg/kg), *Walsura trichostemon* (WT; 100 mg/kg) and lipopolysaccharide.

CHAPTER V

DISCUSSION AND CONCLUSION

Fever is an elevation of body temperature above the normal circadian range as the result of a change in the thermoregulatory center located in the anterior hypothalamus. Fever is not a disease itself, but a manifestation of a number of disease processes. Numerous animal models have shown that survival in the face of infection is enhanced by the production of fever (up to a certain temperature). In human *in vitro* and *in vivo* experiments, fever appears to have a beneficial effect on host defenses including enhancing neutrophil migration, increasing the production of antibacterial substances by neutrophils, and increasing T-cell proliferation. Fever is thought to be produced by several endogenous substances including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α) and prostaglandins (Kuger, 1991; Blatteis and Sehic, 2005; Mihai et al., 2000). The cytokine cascade of fever induction starting from initial stimulation of IL-1 and TNF- α by bacterial products that induces secondary synthesis of IL-6 with subsequent induction of prostaglandin (PG) synthesis in the central nervous system (CNS) and fever. Prostaglandin E_2 (PGE_2), thus induced by these cytokines, is considered to be the proximal, final fever mediator in the preoptic-anterior hypothalamus (POAH) (Aronoff and Neilson, 2001).

Antipyretics such as ASA and other nonsteroidal anti-inflammatory drugs (NSAIDs) reduce fever by depressing inflammatory messages at both peripheral sites of tissue inflammation and within CNS thermoregulation sites. They suppress peripheral production of pyrogenic cytokines including TNF-α and IL-1β while lower the thermoregulatory set point by blocking central COX production of PGE_2 (Aronoff and Neilson, 2001).

LPS is the most potent stimulus known for TNF-α production and release and also increases circulating levels of another pyrogen, IL-1. LPS is unquestionably a clinically important pyrogen. This exogenous pyrogen has been shown to produce fever in laboratory animals such as guinea pigs and rabbits by stimulating the production of endogenous TNF-α (Kuger 1991).

For characterization the antipyretic activity of Ben-Cha-Moon-Yai remedy (BMY) and all five herbal root extracts (AM, OI, DS, DL, WT), the LPS-induced fever model in rats was employed in this study (Santos and Rao, 1998). This study employed ASA as a reference drug. Orally administered ASA, the positive control, significantly attenuated fever in LPS-treated rats at all times tested. This could be due to inhibition of cyclooxygenase (COX) and therefore interference with the cascade of the synthesis of prostaglandins (PGs) which induces fever. BMY (125-500 mg/kg), AM, OI, DS, DL and WT (25-400 mg/kg) suspending in 2% Tween 80 solution were administered orally. The oral administration was chosen in order to imitate the normal consumption of 'Ben-Cha-Moon-Yai', the Thai traditional antipyretic herbal medicine.

The baseline rectal temperature of normal rat and other treatment groups before LPS injection were higher than the usual rectal temperature of rats. The rectal temperature of normal rats was decreased with time and was lower than baseline temperature after 4 hr. This phenomenon might be influenced from several factors, first, the impact of various stressors such as moving the rats within their home cage, transferring rats between holding and test room or isolation of rat which can increase body temperature (T_b) (Dallmann et al., 2006). Since stress can lead to elevations in plasma ACTH, corticosterone and glucose levels and reflects T_b (Groenink et al., 1994) suggesting that long periods of habituation are necessary for accurate baseline temperature (Dallmann et al., 2006). Second, the influence of ambient temperature, this experiment was performed between 0800-1800 h at 25±2^{o}C but the previous experiment was carried out between 0800-1700 h at 28±1^{o}C which is considered the thermoneutral zone for rats (Gordon, 1990). Third, the influence from the investigator including animal handling and drug administration techniques.

In the classic model, the fever condition entails enhanced formation of cytokines such as IL-1β, IFN and TNF-α, and the cytokines increase the synthesis of PGE_2. Aspirin suppresses this response by inhibiting the synthesis of PGE_2 (Vane, 1987). In the recent alternative model, exogenous pyrogens entering the body immediately encounter local mononuclear phagocytes as well as being quickly transported to the liver, their principal storehouse (Küpffer cell, Kc), causing on contact the activation of the complement cascade and the consequent rapid release of PGE_2 by these cells. The

released PGE_2 then stimulates hepatic vagal afferents that convey the pyrogenic message to the POAH, provoking the release of NE, thereby causing successive hyperthermic actions (Blatteis, 2010).

The antipyretic effect of the highest dose of BMY (500 mg/kg) occurred within 1 hr after LPS injection, while other doses of BMY (125 and 250 mg/kg) showed antipyretic effect within 2 hr after LPS injection. The antipyretic effect of BMY (125 and 500 mg/kg) were sustained for up to 7 hr after LPS injection. The highest dose of BMY had the fastest onset of antipyretic action and seemed to have the highest antipyretic efficacy. BMY displayed antipyretic activity in the LPS-induced fever model of rats over 1-7 hr after LPS injection, supporting the view that BMY may be involved in the inhibition of some processes or some substances involving fever. Additional studies are needed to determine the mechanism of BMY actions involved in the inhibition of some of these substances, including IL-1, IL-6, TNF, NF-**κ**B, C3a, C5a, NE, NO and PGs.

Only AM at the dose of 400 mg/kg showed antipyretic activity starting from 2 hr and the effect was sustained for up to 7 hr after LPS injection. This result is consistent with the previous study of Arul et al., (2005) that showed antipyretic activity of all serial extracts of *Aegle marmelos* leaves (50 mg/kg) in mice made hyperthermic by dried yeast injection. Both Thai and Indian traditional systems, the uses of roots of *Aegle marmelos* for curing fever have already been documented. Therefore, this study confirmed traditional claims and provided additional evidence to support the use of roots of *Aegle marmelos* in treating fever.

OI at the doses of 25, 100 and 400 mg/kg showed antipyretic activity starting from 2 hr and the effect was sustained for up to 7 hr after LPS injection. OI 400 mg/kg seemed to have the highest antipyretic efficacy. In Thai traditional medicine, the roots of *Oroxylum indicum* have been used for treating fever without scientific data support. This is the first study that demonstrated the antipyretic activity of the roots of *Oroxylum indicum* in an animal model.

DS did not showed antipyretic activity in the LPS-induced fever model possibly because of the high level of baseline temperature of both control LPS and DS treatment groups. DL showed antipyretic effect only at 2-3 hr after LPS injection suggesting that DL could display a very short duration of antipyretic action when compared with other

herbal roots. There is no document regarding the use of *Dimocarpus longan* or *Dolichandrone serrulata* roots in treating fever in Thai traditional medicine. The result from this study confirmed that both *Dimocarpus longan* and *Dolichandrone serrulata* roots are not potent enough to be used as an antipyretic agent by themselves. However, they might be useful for other indications since Ben-Cha-Moon-Yai remedy has also been used for treating other symptoms including anti-inflammation and antiflatulence by Thai traditional doctors.

All doses of WT except the highest dose showed antipyretic activity starting from 2 or 4 hr after LPS injection and the effect was sustained for up to 7 hr. WT had slower onset of antipyretic action when compared to other herbal roots which may be due to slow absorption of this extract from the gastrointestinal tract. WT 100 mg/kg seemed to have the highest antipyretic efficacy. The highest dose of WT had no antipyretic effect which may be due to high toxicity or minimal absorption of the extract. This is the first study that demonstrated the antipyretic activity of the root of *Walsura trichostemon* in an animal model.

In order to investigate the herbal root extracts that contribute to the antipyretic effect of BMY, active herbal components of BMY at a dose equal to the part in Ben-Cha-Moon-Yai remedy were compared with BMY. The antipyretic effect of BMY 125 mg/kg seemed to be due to OI 25 mg/kg and WT of 25 mg/kg. The antipyretic effect of BMY 250 mg/kg seemed to be due to WT 50 mg/kg. The antipyretic effect of BMY 500 mg/kg seemed to be due to OI 100 mg/kg and WT 100 mg/kg. BMY 500 mg/kg seemed to be the most potent antipyretic agent and more potent than individual components due to additive and/or synergistic effects of some herbal roots in the remedy. This might be a reason why Thai traditional doctors use Ben-Cha-Moon-Yai remedy as an antipyretic agent instead of using individual roots. Although DS and DL showed negligible antipyretic efficacy, they might be included in the remedy in order to reduce toxicity of other roots (if any) or contribute other pharmacological effects that help relieve all symptoms accompanied fever. Ben-Cha-Moon Yai remedy has also been used as an anti-inflammatory agent indicating that some herbal roots in the formula may have other pharmacological effects that are beneficial in treating inflammation.

When comparing between the most effective doses of BMY and individual components. BMY (500 mg/kg), AM (400 mg/kg), OI (400 mg/kg) and WT (100 mg/kg) had comparable antipyretic efficacy. According to the lowest dose used, WT seemed to be the most potent antipyretic agent. This can be concluded that the antipyretic efficacy of BMY is due to the combinations of AM, OI, and WT. AM, OI, and WT at these doses may be used alone as an antipyretic agent or in combination such as 4:4:1 ratio in a new remedy. It is interesting to investigate further the antipyretic efficacy of new remedies which composed of only these three herbal roots in different ratios that have equal or higher efficacy than BMY.

In conclusion, the whole extract of Ben-Cha-Moon-Yai remedy at all doses tested demonstrated anti-pyretic efficacy. The antipyretic effect of BMY may be due to the antipyretic property of AM, OI and WT. This is the first study that helps clarifying the pharmacological action of this herbal remedy and provides additional scientific support for this Thai traditional medicine. Additional studies are required to better understand their potential antipyretic mechanism of action.

FUTURE RESEARCH

The future researches may consist of several objectives as listed below

1. To investigate the antipyretic effects of the combination of the root extracts of AM, OI and WT in different ratios.
2. To investigate the antipyretic effects of the root extract of Ben-Cha-Moon-Yai remedy and each herbal root extracts of Ben-Cha-Moon-Yai remedy in other antipyretic models.
3. To investigate the antipyretic mechanisms of the root extract of Ben-Cha-Moon-Yai remedy.and each herbal root extracts of Ben-Cha-Moon-Yai remedy.
4. To investigate other routes of administration of the root extract of Ben-Cha-Moon-Yai remedy and each herbal root extracts of Ben-Cha-Moon-Yai remedy that might enhance the antipyretic effects.
5. To investigate the anti-inflammatory effects of Ben-Cha-Moon-Yai remedy and various doses of each herbal root extracts of Ben-Cha-Moon-Yai remedy compared with non-steroidal anti-inflammatory drugs.
6. To investigate the anti-inflammatory mechanisms of the root extract of Ben-Cha-Moon-Yai remedy and each herbal root extracts of Ben-Cha-Moon-Yai remedy.
7. To test side effects and toxicity of the root extract of Ben-Cha-Moon-Yai remedy and each herbal root extracts of Ben-Cha-Moon-Yai remedy.

The other studies may provide important clues to help understand the mechanism underlying the antipyretic of each herbal root extracts of Ben-Cha-Moon-Yai remedy and the extract of Ben-Cha-Moon-Yai remedy and further support the use of this Thai traditional medicine in a clinical setting.

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APPENDICES

Appendix A

Certificate of Project Approval

Institutional Animal Care and Use Committee (IACUC)

Faculty of Pharmaceutical Sciences, Chulalongkorn University,

Bangkok, Thailand

Chulalongkorn University Animal Care and Use Committee

Certificate of Project Approval	☐ Original ☐ Renew
Animal Use Protocol No. 10-33-008	**Approval No. 10-33-008**
Protocol Title Antipyretic effect of five herbal root extracts of Bencha-Moon-Yai remedy in rats	
Principal Investigator Pasarapa Towiwat, Ph.D.	
Certification of Institutional Animal Care and Use Committee (IACUC) This project has been reviewed and approved by the IACUC in accordance with university regulations and policies governing the care and use of laboratory animals. The review has followed guidelines documented in Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes edited by the National Research Council of Thailand.	
Date of Approval April 5, 2010	**Date of Expiration** April 5, 2011
Applicant Faculty/Institution Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phyathai Rd., Pathumwan BKK-THAILAND. 10330	
Signature of Chairperson	**Signature of Authorized Official**
Name and Title THONGCHAI SOOKSAWATE, Ph.D. Chairman	**Name and Title** PARKPOOM TENGAMNUAY, Ph.D. Associate Dean (Research and Academic Service)
The official signing above certifies that the information provided on this form is correct. The institution assumes that investigators will take responsibility, and follow university regulations and policies for the care and use of animals. *This approval is subjected to assurance given in the animal use protocol and may be required for future investigations and reviews.*	

Appendix B

Preparation of five herbal root extracts and Thin-Layer Chromatogram of the methanolic extract of five herbal roots of Ben-Cha-Moon-Yai remedy

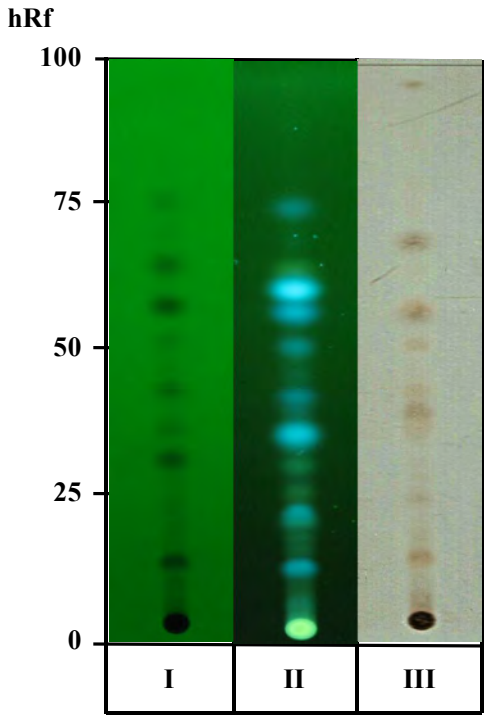
Preparation of five herbal root extracts

Ben-Cha-Moon-Yai remedy consists of five herbal roots of *Aegle marmelos* Linn. Corr. (Ma-tum; AM), *Oroxylum indicum* Linn. Kurz. (Phe-ka; OI), *Dimocarpus longan* Lour. (Lam-yai; DL), *Dolichandrone serrulata* DC. Seem. (Khare-tare; DS) and *Walsura trichostemon* Miq. (Kad-lin: WT). They were collected from Nakonrachasima province of Thailand and authenticated by Ruangrungsi. The voucher and number of specimens were deposited at the College of Public Health Sciences, Chulalongkorn University, Thailand. All five roots species were dried under shade and grinded to coarse powders. Each powder of five roots was macerated in the absolute ethanol in a closed conical flask for 24 hours. The marc after filtration was dried and further macerated with water for 24 hours. The ethanol extracts were evaporated to dryness under vacuum. The water extracts were lyophilized to dryness. The extracts yield were weighed, recorded and stored at -20 °C. The Ben-Cha-Moon-Yai remedy (BMY) was prepared by mixing each extract in the quantity equivalent to the traditional remedy preparation. The extract was prepared by Ms. Rawiwan Manohan, College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand.

Thin-layer chromatographic identification

One gram of each herbal root powder of *Aegle marmelos* Linn. Corr., *Oroxylum indicum* Linn. Kurz., *Dimocarpus longan* Lour., *Dolichandrone serrulata* DC. Seem. and *Walsura trichostemon* Miq. was macerate with 20 ml of methanol for 12 hours, filter and evaporate to dryness. Dissolve the residue in 0.5 ml of methanol. Apply 10 µl to the thin-layer chromatographic plate, using siliga gel 60 F_{254} as the coating substance. The plate was removed and allowed to dry and observed for the produced spots under short-wave (254 nm) and long-wave (366 nm) ultraviolet light. The plate was sprayed with the mixture solution of 10% sulfuric acid reagent (conc. sulfuric acid 10 ml in methanol 90 ml). The plate was then placed in the hot air oven at 105°C for 5 min. The thin-layer chromatograms of all five herbal root extracts were done by Ms. Rawiwan Manohan, College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand

Fiugure 24 Thin-layer chromatogram of the methanolic extract of the root of *Aegle marmelos* Corr.



Solvent system

Toluene : Ethyl acetate 75:25

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid *, **

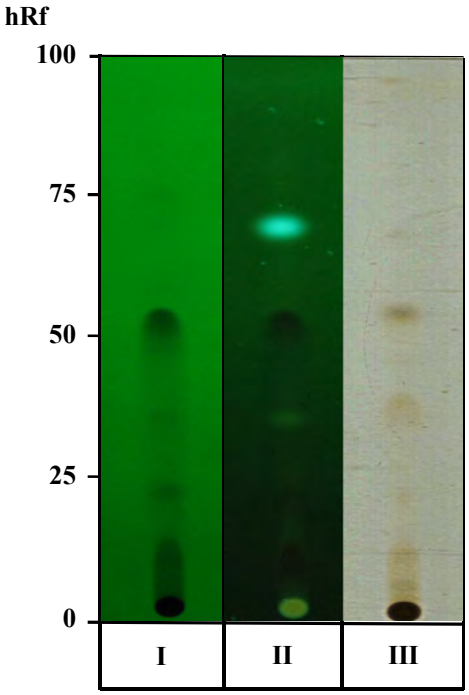
*10% sulfuric acid reagent

Preparation: conc. sulfuric acid 10 ml. in methanol 90 ml.

**Spot color Development

Heat the plate at 120 $^{\circ}$ C for 10 minutes after sprayed.

Fiugure 25 Thin-layer chromatogram of the methanolic extract of the root of *Oroxylum indicum* Vent.



Solvent system

Toluene : Ethyl acetate 75:25

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid*,**

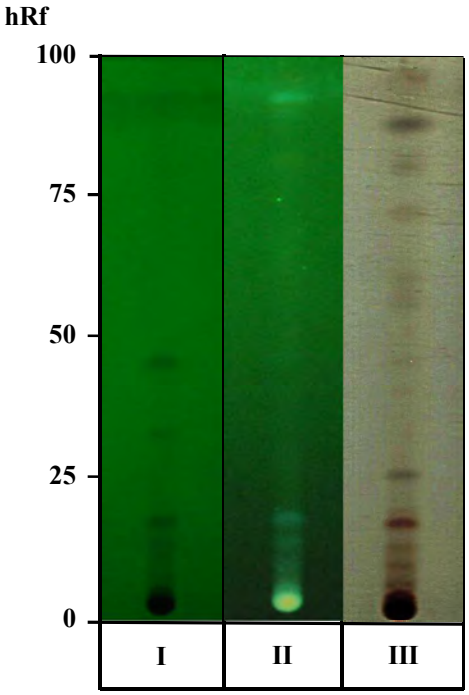
*10% sulfuric acid reagent

Preparation: conc. sulfuric acid 10 ml. in methanol 90 ml.

**Spot color Development

Heat the plate at 120 $^{\circ}$ C for 10 minutes after sprayed.

Fiugure 26 Thin-layer chromatogram of the methanolic extract of the root of *Dolichandrone serrulata* (DC) Seem.



Solvent system

Chloroform : Methanol 9 : 1

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid*,**

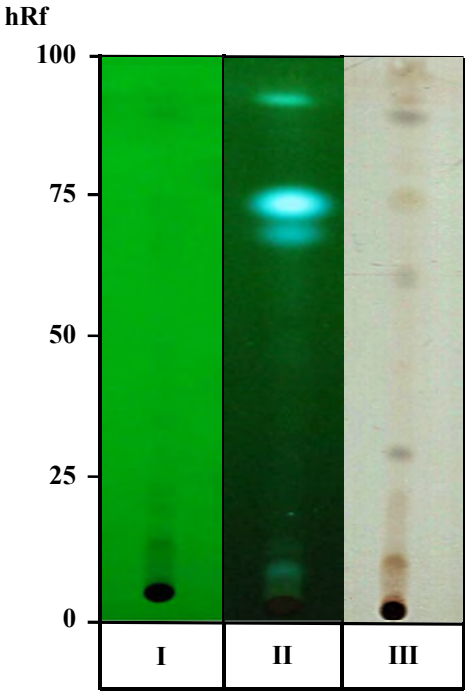
*10% sulfuric acid reagent

Preparation: conc. sulfuric acid 10 ml. in methanol 90 ml.

**Spot color Development

Heat the plate at 120 $^{\circ}$ C for 10 minutes after sprayed.

Fiugure 27 Thin-layer chromatogram of the methanolic extract of the root of *Dimocarpus longan* Lour.



Solvent system

Chloroform : Methanol 9 : 1

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid*,**

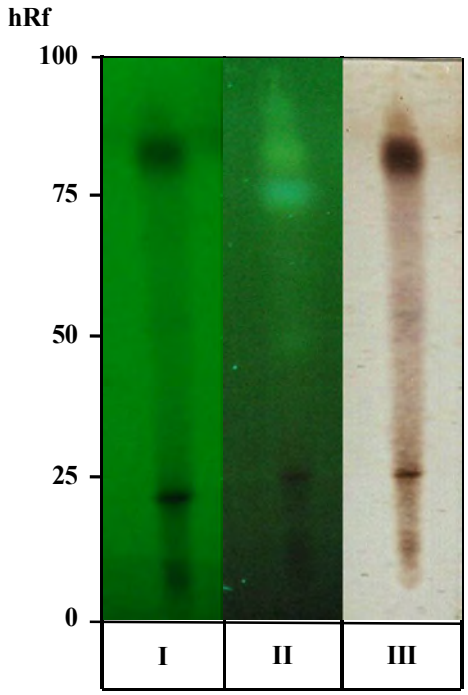
*10% sulfuric acid reagent

Preparation: conc. sulfuric acid 10 ml. in methanol 90 ml.

**Spot color Development

Heat the plate at 120 $^{\circ}$ C for 10 minutes after sprayed.

Fiugure 28 Thin-layer chromatogram of the methanolic extract of the root of *Walsura trichostemon* Miq



Solvent system

n-butanol : acetic acid : water 4 : 1 : 5

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid*,**

*10% sulfuric acid reagent

Preparation: conc. sulfuric acid 10 ml. in methanol 90 ml.

**Spot color Development

Heat the plate at 120 $^{\circ}$ C for 10 minutes after sprayed.

Appendix C

Data of Lipopolysaccharide-induced Fever in Rats

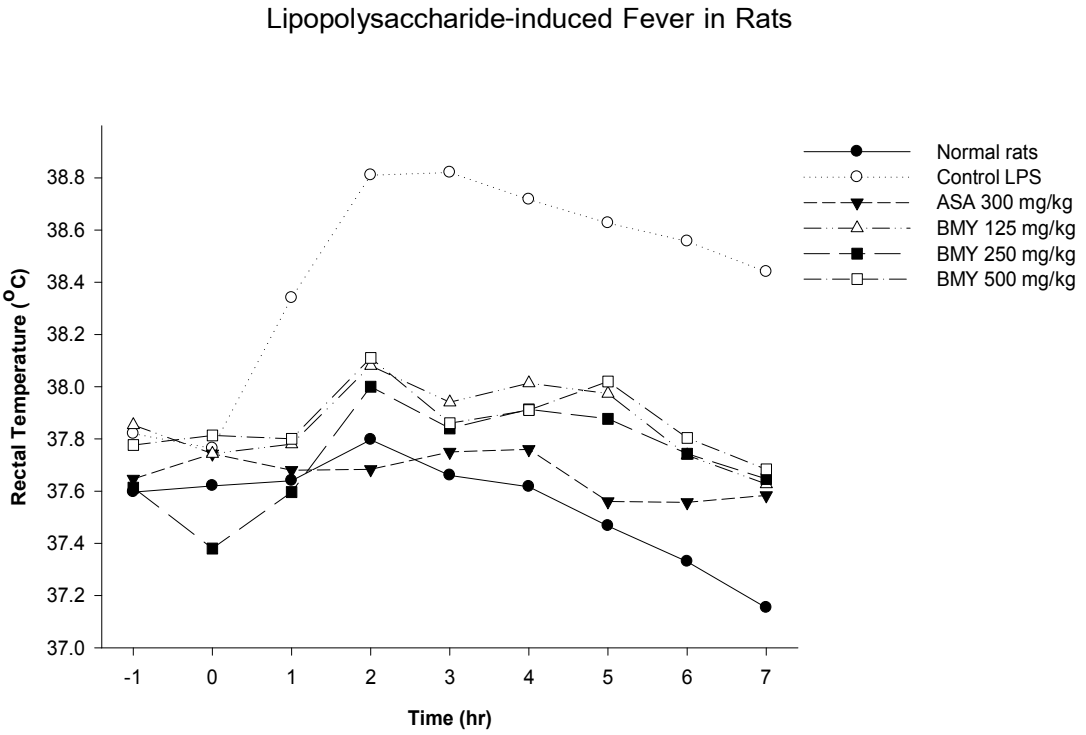


Figure 29 Changes in rectal temperature after oral administration of 2% Tween 80 (control), acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr prior to LPS. N=6 for all groups. *p<0.05 significantly different compared to control values at the corresponding hour.

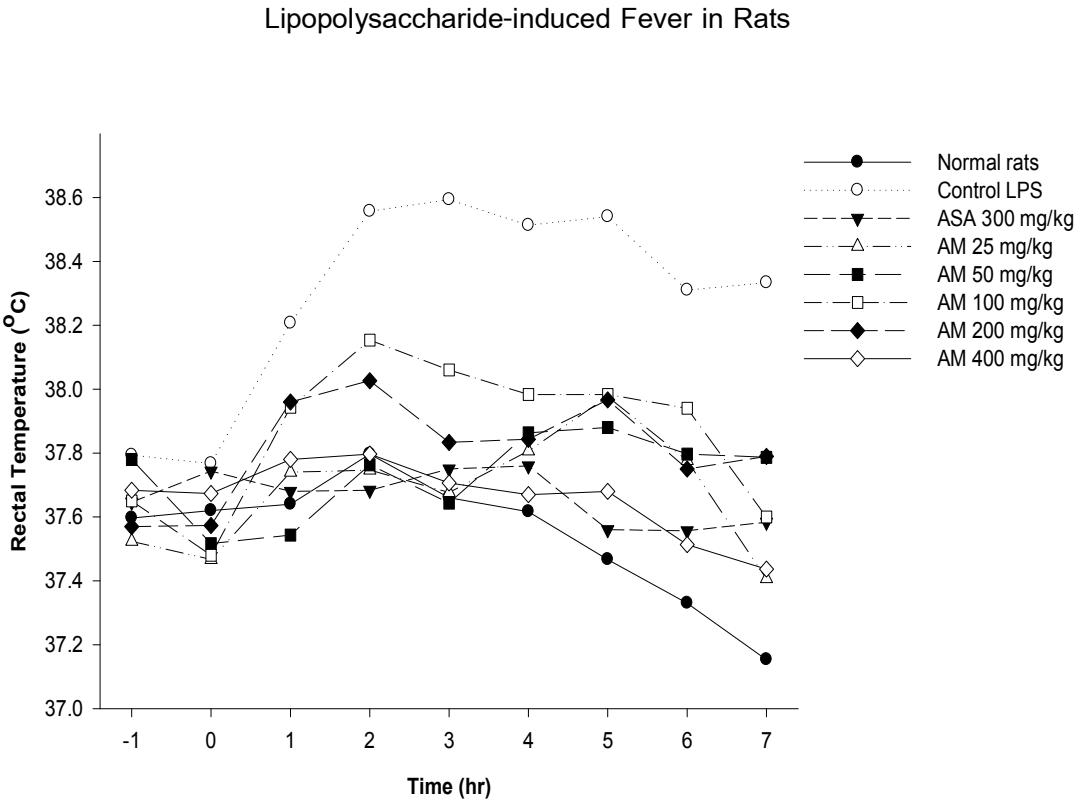


Figure 30 Changes in rectal temperature after oral administration of 2% Tween 80 (control), acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Aegle marmelos* (AM; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr prior to LPS, N=6 for all groups. *p<0.05 significantly different compared to control values at the corresponding hour.

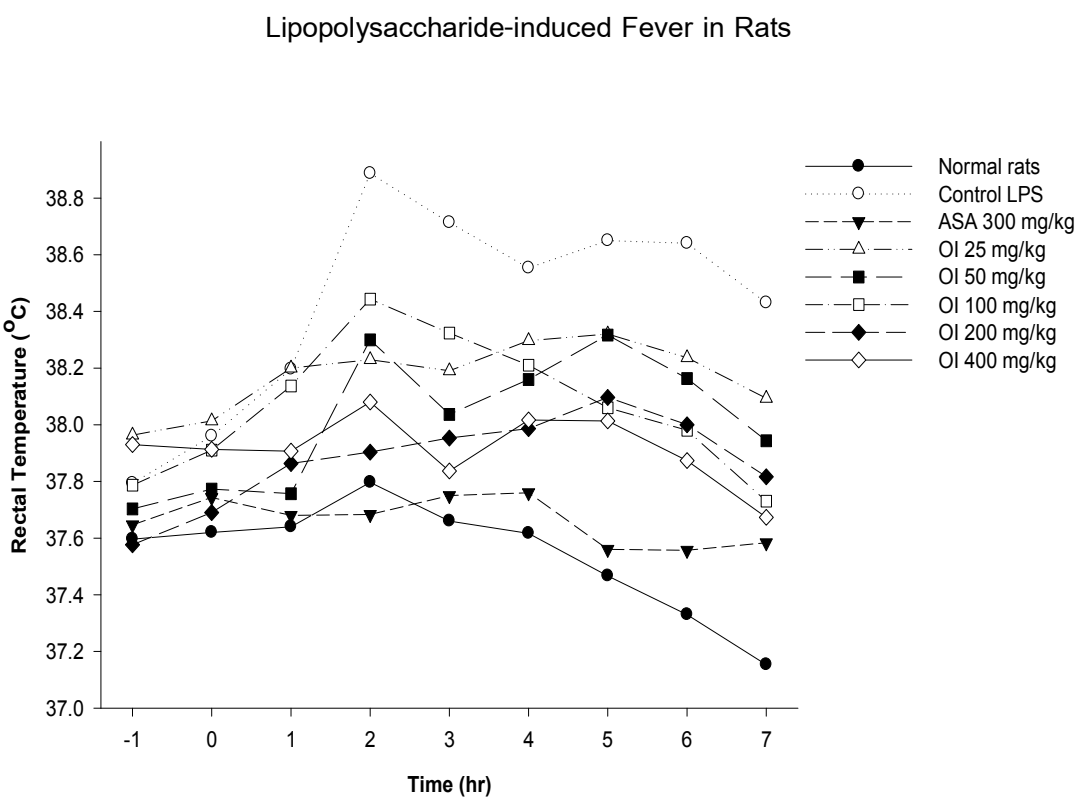


Figure 31 Changes in rectal temperature after oral administration of 2% Tween 80 (control), acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Oroxylum indicum* (OI; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr prior to LPS. N=6 for all groups. *$p<0.05$ significantly different compared to control values at the corresponding hour.

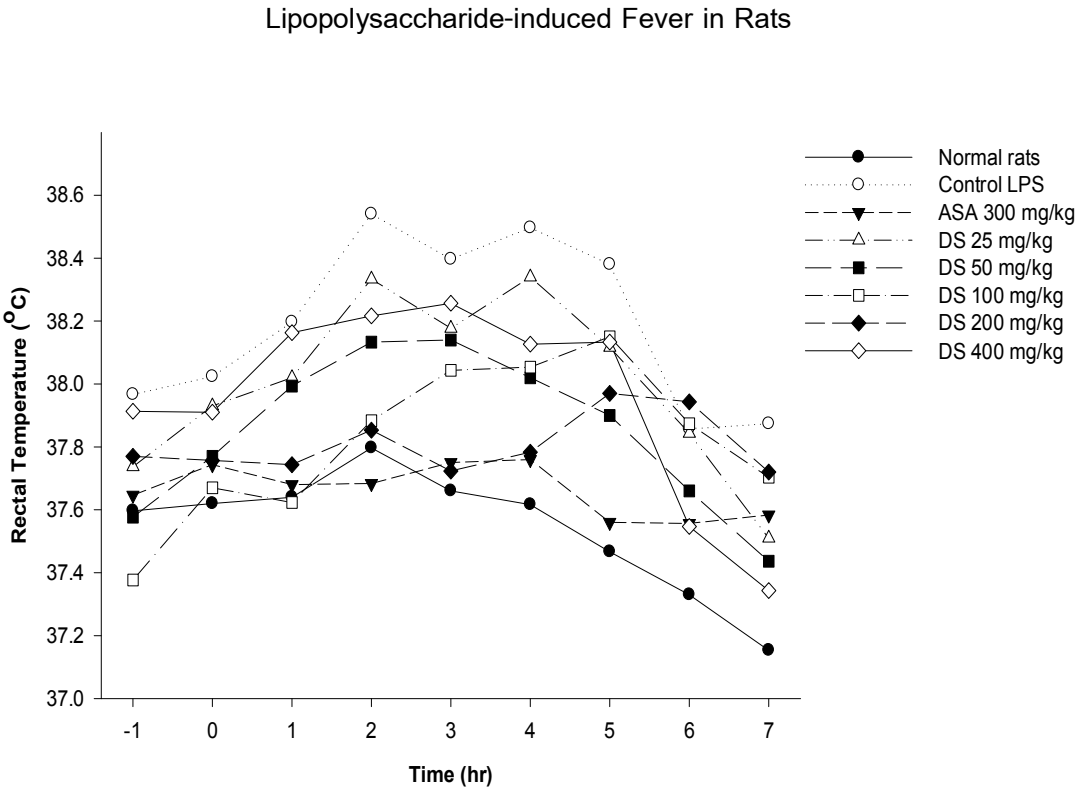


Figure 32 Changes in rectal temperature after oral administration of 2% Tween 80 (control), acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Dolichandrone serrulata* (DS; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr prior to LPS. N=6 for all groups. *$p<0.05$ significantly different compared to control values at the corresponding hour.

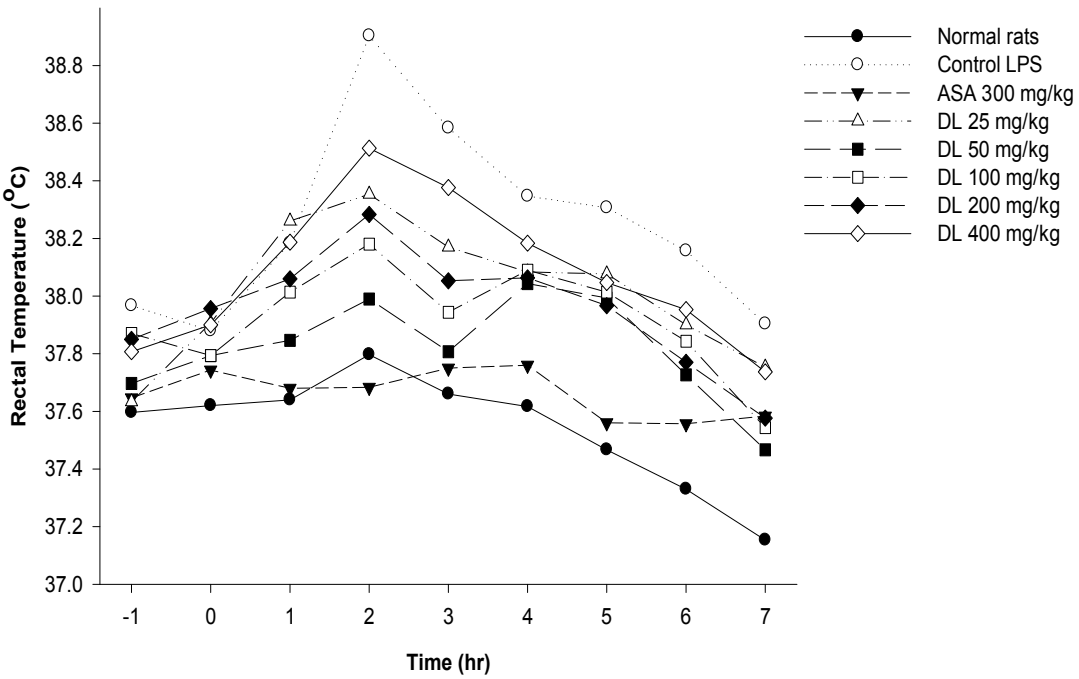


Figure 33 Changes in rectal temperature after oral administration of 2% Tween 80 (control), acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Dimocarpus longan* (DL; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr prior to LPS, N=6 for all groups. *$p<0.05$ significantly different compared to control values at the corresponding hour.

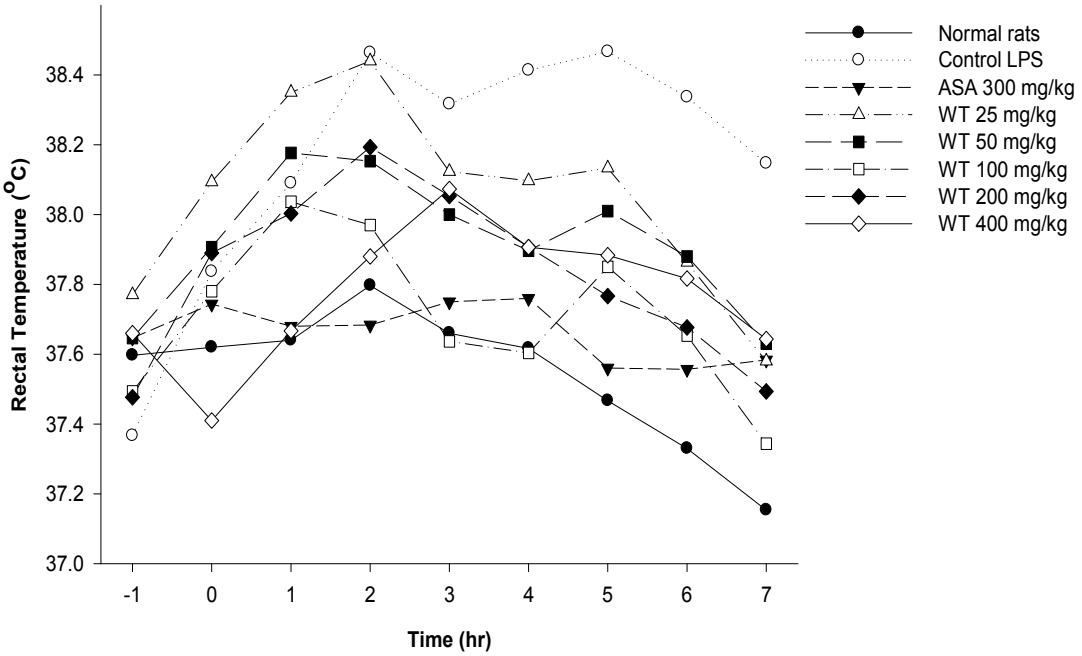


Figure 34 Changes in rectal temperature after oral administration of 2% Tween 80 (control), acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of *Walsura trichostemon* (WT; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr prior to LPS. N=6 for all groups. *$p<0.05$ significantly different compared to control values at the corresponding hour.

Table 4 Effect of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg) on lipopolysaccharide-induced fever in rats.

Treatments	Rectal Temperature (°C) before and after LPS injection								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normothermic rats[a]	37.59±0.11	37.62±0.10	37.64±0.21	37.79±0.14	37.66±0.14	37.61±0.07	37.46±0.15	37.33±0.15	37.15±0.15
Control LPS[b]	37.82±0.10	37.76±0.93	38.34±0.04	38.81±0.15[#]	38.82±0.10[##]	38.71±0.07[##]	38.62±0.06[##]	38.55±0.74[##]	38.44±0.10[##]
ASA 300 mg/kg	37.64±0.21	37.74±0.21	37.68±0.23	37.68±0.22*	37.75±0.17*	37.76±0.21*	37.56±0.19*	37.55±0.23*	37.58±0.24*
BMY 125 mg/kg	37.85±0.88	37.74±0.11	37.78±0.08	38.08±0.13*	37.94±0.13*	38.01±0.12*	37.97±0.06*	37.73±0.13*	37.62±0.12*
BMY 250 mg/kg	37.61±0.92	37.38±0.11	37.59±0.21	38.00±0.18*	37.84±0.14*	37.91±0.19*	37.87±0.17*	37.74±0.16*	37.64±0.16*
BMY 500 mg/kg	37.77±0.11	37.81±0.17	37.80±0.19	38.11±0.83	37.86±0.15*	37.91±0.12*	38.02±0.05*	37.87±0.87*	37.72±0.10*

Each value represents mean ± S.E.M., N=6 for all groups. [a]Normothermic rats received 0.9% NSS. [b]Control LPS received 2% Tween 80 solution. [#]$p<0.05$ and [##]$p<0.01$ significantly different compared to normothermic rat values for the corresponding hour. *$p<0.05$ significantly different compared to control LPS values at the corresponding hour.

Table 5 Effect of the root extract of *Aegle marmelos* (AM; 25-400 mg/kg) on lipopolysaccharide-induced fever in rats.

Treatments	Rectal Temperature (°C) before and after LPS injection								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normothermic rats[a]	37.59±0.11	37.62±0.10	37.64±0.21	37.79±0.14	37.66±0.14	37.61±0.07	37.46±0.15	37.33±0.15	37.15±0.15
Control LPS[b]	37.79±0.09	37.77±0.08	38.21±0.08	38.56±0.16[#]	38.59±0.11[##]	38.51±0.18[#]	38.54±0.15[##]	38.31±0.17[#]	38.33±0.15[##]
ASA 300 mg/kg	37.64±0.21	37.74±0.21	37.6±0.23	37.68±0.22*	37.75±0.17*	37.76±0.21*	37.56±0.19*	37.55±0.23*	37.58±0.24*
AM 25 mg/kg	37.52±0.22	37.47±0.17	37.74±0.10	37.75±0.04*	37.68±0.15*	37.81±0.19	37.98±0.04	37.78±0.13	37.41±0.12*
AM 50 mg/kg	37.78±0.07	37.52±0.16	37.54±0.08*	37.76±0.06*	37.64±0.14*	37.86±0.20	37.88±0.15*	37.80±0.19	37.79±0.21
AM 100 mg/kg	37.65±0.12	37.48±0.10	37.94±0.09	38.15±0.11	38.06±0.08	37.98±0.06	37.98±0.09	37.94±0.07	37.60±0.09
AM 200 mg/kg	37.57±0.13	37.57±0.19	37.96±0.05	38.03±0.10	37.83±0.09*	37.84±0.10	37.97±0.12	37.75±0.12	37.79±0.14
AM 400 mg/kg	37.68±0.14	37.67±0.10	37.78±0.13	37.80±0.14*	37.71±0.13*	37.67±0.12*	37.68±0.09*	37.51±0.09*	37.44±0.15*

Each value represents mean ± S.E.M., N=6 for all groups. [a]Normothermic rats received 0.9% NSS. [b]Control LPS received 2% Tween 80 solution. [#]$p<0.05$ and [##]$p<0.001$ significantly different compared to normothermic rat values for the corresponding hour.*$p<0.05$ significantly different compared to control LPS values at the corresponding hour.

Table 6 Effect of the root extract of *Oroxylum indicum* (OI; 25-400 mg/kg) on lipopolysaccharide-induced fever in rats.

Treatments	Rectal Temperature (°C) before and after LPS injection								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normothermic rats[a]	37.59±0.11	37.62±0.10	37.64±0.21	37.79±0.14	37.66±0.14	37.61±0.07	37.46±0.15	37.33±0.15	37.15±0.15
Control LPS[b]	37.79±0.09	37.96±0.09	38.20±0.12	38.89±0.08[##]	38.71±0.03[##]	38.55±0.05[##]	38.65±0.09[##]	38.64±0.14[##]	38.43±0.13[##]
ASA 300 mg/kg	37.64±0.21	37.74±0.21	37.68±0.23	37.68±0.22*	37.75±0.17*	37.76±0.21*	37.56±0.19*	37.55±0.23*	37.58±0.24*
OI 25 mg/kg	37.96±0.12	38.01±0.05	38.20±0.09	38.23±0.13*	38.19±0.11	38.30±0.09	38.32±0.05	38.24±0.13	38.09±0.13
OI 50 mg/kg	37.70±0.15	37.77±0.12	37.76±0.11	38.30±0.08	38.04±0.06*	38.16±0.13	38.32±0.13	38.16±0.19	37.94±0.16
OI 100 mg/kg	37.79±0.09	37.91±0.17	38.14±0.20	38.44±0.12	38.32±0.09	38.21±0.10	38.06±0.05*	37.98±0.04	37.73±0.13*
OI 200 mg/kg	37.58±0.17	37.69±0.18	37.86±0.14	37.90±0.14*	37.95±0.16*	37.99±0.13	38.10±0.05*	38.00±0.08	37.82±0.10
OI 400 mg/kg	37.93±0.08	37.91±0.10	37.91±0.12	38.08±0.10*	37.84±0.09*	38.02±0.13	38.01±0.14*	37.87±0.11*	37.67±0.10*

Each value represents mean ± S.E.M. N=6 for all groups. [a]Normothermic rats received 0.9% NSS. [b]Control LPS received 2% Tween 80 solution. [##]$p<0.001$ significantly different compared to normothermic rat values for the corresponding hour. *$p<0.05$ significantly different compared to control LPS values at the corresponding hour.

Table 7 Effect of the root extract of *Dolichandrone serrulata* (DS; 25-400 mg/kg) on lipopolysaccharide-induced fever in rats.

Treatments	Rectal Temperature (°C) before and after LPS injection								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normothermic rats[a]	37.59±0.11	37.62±0.10	37.64±0.21	37.79±0.14	37.66±0.14	37.61±0.07	37.46±0.15	37.33±0.15	37.15±0.15
Control LPS[b]	37.97±0.12	38.02±0.11	38.20±0.11	38.54±0.11[#]	38.40±0.06[#]	38.50±0.09[#]	38.38±0.15[#]	37.86±0.13	37.87±0.18[#]
ASA 300 mg/kg	37.64±0.21	37.74±0.21	37.68±0.23	37.68±0.22*	37.75±0.17	37.76±0.21*	37.56±0.19*	37.55±0.23	37.58±0.24
DS 25 mg/kg	37.74±0.06	37.93±0.11	38.02±0.08	38.33±0.08	38.18±0.10	38.34±0.09	38.12±0.13	37.84±0.11	37.51±0.08
DS 50 mg/kg	37.58±0.18	37.77±0.04	37.99±0.17	38.13±0.06	38.14±0.10	38.02±0.15	37.90±0.11	37.66±0.06	37.44±0.07
DS 100 mg/kg	37.38±0.28	37.67±0.16	37.62±0.10	37.88±0.17	38.04±0.17	38.05±0.09	38.15±0.08	37.87±0.10	37.70±0.06
DS 200 mg/kg	37.71±0.14	37.71±0.07	37.76±0.16	37.85±0.10*	37.71±0.23	37.73±0.16*	37.87±0.14	37.83±0.15	37.63±0.15
DS 400 mg/kg	37.91±0.15	37.91±0.15	38.16±0.15	38.22±0.17	38.26±0.14	38.13±0.07	38.13±0.14	37.55±0.14	37.34±0.17

Each value represents mean ± S.E.M., N=6 for all groups. [a]Normothermic rats received 0.9% NSS. [b]Control LPS received 2% Tween 80 solution. [#]$p<0.05$ significantly different compared to normothermic rat values for the corresponding hour. *$p<0.05$ significantly different compared to control LPS values at the corresponding hour.

Table 8 Effect of the root extract of *Dimocarpus longan* (DL; 25-400 mg/kg) on lipopolysaccharide-induced fever in rats.

Treatments	Rectal Temperature (°C) before and after LPS injection								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normothermic rats[a]	37.59±0.11	37.62±0.10	37.64±0.21	37.79±0.14	37.66±0.14	37.61±0.07	37.46±0.15	37.33±0.15	37.15±0.15
Control LPS[b]	37.97±0.12	37.88±0.11	38.18±0.11	38.90±0.11$^{\#\#}$	38.58±0.06$^{\#\#}$	38.35±0.09$^{\#}$	38.31±0.15$^{\#}$	38.16±0.13$^{\#}$	37.90±0.18$^{\#}$
ASA 300 mg/kg	37.64±0.21	37.74±0.21	37.68±0.23	37.68±0.22*	37.75±0.17*	37.76±0.21	37.56±0.19*	37.55±0.23	37.58±0.24
DL 25 mg/kg	37.63±0.06	37.91±0.11	38.26±0.08	38.35±0.08	38.17±0.10	38.08±0.09	38.08±0.13	37.90±0.11	37.75±0.08
DL 50 mg/kg	37.70±0.18	37.79±0.04	37.85±0.17	37.99±0.06*	37.81±0.10*	38.04±0.15	37.99±0.11	37.73±0.06	37.47±0.07
DL 100 mg/kg	37.87±0.28	37.79±0.16	38.01±0.10	38.18±0.17*	37.94±0.17*	38.09±0.09	38.01±0.08	37.84±0.10	37.54±0.06
DL 200 mg/kg	37.85±0.14	37.96±0.07	38.06±0.16	38.28±0.10	38.05±0.23	38.06±0.16	37.97±0.14	37.77±0.15	37.58±0.15
DL 400 mg/kg	37.81±0.15	37.90±0.15	38.19±0.15	38.51±0.17	38.38±0.14	38.18±0.07	38.05±0.14	37.95±0.14	37.74±0.17

Each value represents mean ± S.E.M., N=6 for all groups. [a]Normothermic rats received 0.9% NSS. [b]Control LPS received 2% Tween 80 solution. $^{\#}p<0.05$ and $^{\#\#}p<0.001$ significantly different compared to normothermic rat values for the corresponding hour.*$p<0.05$ significantly different compared to control LPS values at the corresponding hour.

Table 9 Effect of the root extract of *Walsura trichostemon* (WT; 25-400 mg/kg) on lipopolysaccharide-induced fever in rats

Treatments	Rectal Temperature (°C) before and after LPS injection								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normothermic rats[a]	37.59±0.11	37.62±0.10	37.64±0.21	37.79±0.14	37.66±0.14	37.61±0.07	37.46±0.15	37.33±0.15	37.15±0.15
Control LPS[b]	37.37±0.12	37.84±0.06	38.09±0.07	38.46±0.1[#]	38.32±0.18	38.41±0.1[#]	38.47±0.2[#]	38.34±0.2[#]	38.15±0.1[#]
ASA 300 mg/kg	37.64±0.21	37.74±0.21	37.68±0.23	37.68±0.2*	37.75±0.17	37.76±0.21	37.56±0.1*	37.55±0.23	37.58±0.24
WT 25 mg/kg	37.77±0.15	38.09±0.09	38.35±0.06	38.44±0.07	38.12±0.14	38.10±0.13	38.13±0.12	37.86±0.08	37.58±0.10
WT 50 mg/kg	37.65±0.12	37.91±0.07	38.18±0.09	38.15±0.09	38.00±0.07	37.90±0.08	38.01±0.10	37.88±0.6	37.63±0.15
WT 100mg/kg	37.49±0.17	37.78±0.09	38.04±0.07	37.97±0.12	37.64±0.12	37.60±0.1*	37.85±0.11	37.65±0.11	37.34±0.0*
WT 200mg/kg	37.48±0.21	37.89±0.09	38.00±0.10	38.19±0.11	38.05±0.19	37.91±0.17	37.77±0.14	37.68±0.18	37.49±0.20
WT 400mg/kg	37.66±0.19	37.41±0.16	37.67±0.16	37.88±0.13	38.07±0.30	37.91±0.15	37.88±0.12	37.82±0.15	37.64±0.14

Each value represents mean ± S.E.M., N=6 for all groups. [a]Normothermic rats received 0.9% NSS. [b]Control LPS received 2% Tween 80 solution. [#]$p<0.05$ significantly different compared to normothermic rat values for the corresponding hour. *$p<0.05$ significantly different compared to control LPS values at the corresponding hour.

Table 10 Effect of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg) on lipopolysaccharide-induced fever in rats.

Treatments	Rectal Temperature change (°C) before and after LPS injection						
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normothermic rats[a]	0.02	0.18	0.04	0.00	-0.15	-0.29	-0.47
Control LPS[b]	0.58	1.05	1.06	0.95	0.86	0.79	0.68
ASA 300 mg/kg	-0.06	-0.06	0.01	0.02	-0.18	-0.19	-0.16
BMY 125 mg/kg	0.04	0.34	0.20	0.27	0.23	-0.01	-0.12
BMY 250 mg/kg	0.22	0.62	0.46	0.53	0.50	0.36	0.27
BMY 500 mg/kg	-0.01	0.30	0.05	0.10	0.21	-0.01	-0.13

Each value represents mean ± S.E.M., N=6 for all groups. [a]Normothermic rats received 0.9% NSS. [b]Control LPS received 2% Tween 80 solution.

Table 11 Effect of the root extract of *Aegle marmelos* (AM; 25-400 mg/kg) on lipopolysaccharide-induced fever in rats.

Treatments	Rectal Temperature change (°C) before and after LPS injection						
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normothermic rats[a]	0.02	0.18	0.04	0.00	-0.15	-0.29	-0.47
Control LPS[b]	0.44	0.79	0.83	0.75	0.77	0.54	0.57
ASA 300 mg/kg	-0.06	-0.06	0.01	0.02	-0.18	-0.19	-0.16
AM 25 mg/kg	0.27	0.28	0.21	0.34	0.51	0.31	-0.06
AM 50 mg/kg	0.03	0.25	0.13	0.35	0.36	0.28	0.27
AM 100 mg/kg	0.46	0.67	0.58	0.50	0.50	0.46	0.12
AM 200 mg/kg	0.39	0.45	0.26	0.27	0.39	0.18	0.22
AM 400 mg/kg	0.11	0.12	0.03	0.00	0.01	-0.16	-0.24

Each value represents mean ± S.E.M., N=6 for all groups. [a]Normothermic rats received 0.9% NSS. [b]Control LPS received 2% Tween 80 solution.

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Table 12 Effect of the root extract of *Oroxylum indicum* (OI; 25-400 mg/kg) on lipopolysaccharide-induced fever in rats.

Treatments	Rectal Temperature change (°C) before and after LPS injection						
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normothermic rats[a]	0.02	0.18	0.04	0.00	-0.15	-0.29	-0.47
Control LPS[b]	0.24	0.93	0.75	0.59	0.69	0.68	0.47
ASA 300 mg/kg	-0.06	-0.06	0.01	0.02	-0.18	-0.19	-0.16
OI 25 mg/kg	0.19	0.22	0.18	0.28	0.31	0.22	0.08
OI 50 mg/kg	-0.02	0.53	0.26	0.39	0.54	0.39	0.17
OI 100 mg/kg	0.23	0.53	0.41	0.30	0.15	0.07	-0.18
OI 200 mg/kg	0.17	0.21	0.26	0.30	0.41	0.31	0.13
OI 400 mg/kg	-0.01	0.17	-0.08	0.10	0.10	-0.04	-0.24

Each value represents mean ± S.E.M., N=6 for all groups. [a]Normothermic rats received 0.9% NSS. [b]Control LPS received 2% Tween 80 solution.

Table 13 Effect of the root extract of *Dolichandrone serrulata* (DS; 25-400 mg/kg) on lipopolysaccharide-induced fever in rats.

Treatments	Rectal Temperature change (°C) before and after LPS injection						
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normothermic rats[a]	0.02	0.18	0.04	0.00	-0.15	-0.29	-0.47
Control LPS[b]	0.17	0.52	0.37	0.47	0.36	-0.17	-0.15
ASA 300 mg/kg	-0.06	-0.06	0.01	0.02	-0.18	-0.19	-0.16
DS 25 mg/kg	0.09	0.40	0.25	0.41	0.19	-0.09	-0.42
DS 50 mg/kg	0.22	0.36	0.37	0.25	0.13	-0.11	-0.33
DS 100 mg/kg	-0.05	0.21	0.37	0.38	0.48	0.20	0.03
DS 200 mg/kg	-0.01	0.10	-0.03	0.03	0.21	0.19	-0.04
DS 400 mg/kg	0.25	0.31	0.35	0.22	0.22	-0.36	-0.57

Each value represents mean ± S.E.M., N=6 for all groups. [a]Normothermic rats received 0.9% NSS. [b]Control LPS received 2% Tween 80 solution.

Table 14 Effect of the root extract of *Dimocarpus longan* (DL; 25-400 mg/kg) on lipopolysaccharide-induced fever in rats.

Treatments	Rectal Temperature change (°C) before and after LPS injection						
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normothermic ratsa	0.02	0.18	0.04	0.00	-0.15	-0.29	-0.47
Control LPSb	0.30	1.02	0.70	0.47	0.43	0.28	0.02
ASA 300 mg/kg	-0.06	-0.06	0.01	0.02	-0.18	-0.19	-0.16
DL 25 mg/kg	0.35	0.45	0.26	0.18	0.17	-0.01	-0.15
DL 50 mg/kg	0.05	0.20	0.01	0.25	0.20	-0.07	-0.33
DL 100 mg/kg	0.22	0.39	0.15	0.30	0.22	0.05	- 0.25
DL 200 mg/kg	0.10	0.33	0.10	0.11	0.01	-0.19	-0.38
DL 400 mg/kg	0.29	0.61	0.48	0.28	0.15	0.05	-0.16

Each value represents mean ± S.E.M., N=6 for all groups. [a]Normothermic rats received 0.9% NSS. [b]Control LPS received 2% Tween 80 solution.

Table 15 Effect of the root extract of *Walsura trichostemon* (WT; 25-400 mg/kg) on lipopolysaccharide-induced fever in rats

Treatments	Rectal Temperature change (°C) before and after LPS injection						
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normothermic rats[a]	0.02	0.18	0.04	0.00	-0.15	-0.29	-0.47
Control LPS[b]	0.25	0.63	0.48	0.58	0.63	0.50	0.31
ASA 300 mg/kg	-0.06	-0.06	0.01	0.02	-0.18	-0.19	-0.16
WT 25 mg/kg	0.26	0.35	0.03	0.00	0.04	-0.23	-0.51
WT 50 mg/kg	0.27	0.25	0.09	-0.01	0.10	-0.03	-0.28
WT 100mg/kg	0.26	0.19	-0.14	-0.18	0.07	-0.13	-0.44
WT 200mg/kg	0.11	0.30	0.16	0.02	-0.12	-0.21	-0.40
WT 400mg/kg	0.26	0.47	0.66	0.50	0.47	0.41	0.23

Each value represents mean ± S.E.M., N=6 for all groups. [a]Normothermic rats received 0.9% NSS. [b]Control LPS received 2% Tween 80 solution.

Table 16 Effect of 2% Tween 80 (10 ml/kg, p.o.) and NSS (10 mg/kg, i.m.) on normal rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.56	37.20	37.52	38.02	37.74	37.64	38.06	36.80	36.54
2	37.58	37.60	37.44	37.56	37.18	37.36	36.92	36.94	37.68
3	37.36	37.72	38.00	37.78	37.52	37.78	37.30	37.30	37.00
4	37.52	37.82	38.14	38.04	38.12	37.66	37.50	37.74	37.14
5	37.40	37.50	36.72	37.20	37.44	37.46	37.48	37.66	37.32
6	38.16	37.88	38.02	38.18	37.96	37.80	37.54	37.54	37.24
average	37.60	37.62	37.64	37.80	37.66	37.62	37.47	37.33	37.15

Table 17 Effect of ASA (300 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	36.78	36.98	37.84	36.74	37.30	37.48	37.14	36.80	36.88
2	37.86	37.50	37.64	37.46	37.54	37.16	37.28	37.20	37.00
3	38.04	38.48	38.30	38.02	37.78	37.86	38.12	37.50	38.18
4	37.22	38.06	37.40	37.52	37.40	37.30	37.00	37.46	37.66
5	38.06	37.44	36.70	38.10	38.04	38.42	37.80	38.46	38.36
6	37.92	38.00	38.20	38.26	38.44	38.34	38.02	37.92	37.42
average	37.65	37.74	37.68	37.68	37.75	37.76	37.56	37.56	37.58

Table 18 Effect of 2% Tween 80 (10 mg/kg, p.o.) in BMY group on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.64	37.50	38.38	38.10	38.50	38.66	38.54	38.58	38.66
2	38.06	37.80	38.34	39.06	39.12	38.70	38.68	38.52	38.04
3	37.42	37.94	38.40	38.70	38.64	38.58	38.66	38.72	38.52
4	37.78	37.66	38.48	39.02	38.84	38.76	38.52	38.38	38.46
5	38.02	37.58	38.26	39.04	38.72	38.56	38.48	38.34	38.22
6	38.00	38.10	38.18	38.94	39.10	39.04	38.88	38.80	38.74
average	37.82	37.76	38.34	38.81	38.82	38.72	38.63	38.56	38.44

Table 19 Effect of 2% Tween 80 (10 mg/kg, p.o.) in AM group on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.24	37.26	38.30	37.98	38.12	38.00	37.70	37.74	37.78
2	37.78	37.80	38.10	38.82	38.54	38.60	39.00	39.12	38.70
3	37.48	37.64	37.92	38.26	38.56	38.92	38.72	38.24	38.40
4	37.78	37.66	38.48	39.02	38.62	38.56	38.40	38.28	38.46
5	37.98	38.08	38.18	38.20	38.24	37.90	37.90	37.80	37.68
6	38.10	37.92	38.18	38.94	39.10	39.04	38.68	38.24	38.10
average	37.79	37.77	38.21	38.56	38.59	38.51	38.54	38.31	38.33

Table 20 Effect of 2% Tween 80 (10 mg/kg, p.o.) in OI group on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.78	37.66	38.48	39.02	38.84	38.56	38.40	38.28	38.46
2	37.42	37.94	38.40	38.70	38.64	38.62	38.66	38.72	38.52
3	38.08	38.14	38.42	38.84	38.68	38.72	38.74	38.86	38.64
4	37.76	37.88	37.70	38.68	38.60	38.42	38.72	38.64	38.46
5	37.78	38.32	38.10	38.82	38.74	38.60	39.00	39.12	38.70
6	37.94	37.82	38.08	39.26	38.78	38.40	38.38	38.22	37.80
average	37.79	37.96	38.20	38.89	38.71	38.55	38.65	38.64	38.43

Table 21 Effect of 2% Tween 80 (10 mg/kg, p.o.) in DS group on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.44	37.98	38.54	38.52	38.48	38.42	38.42	37.82	38.02
2	38.12	38.00	38.16	38.78	38.22	38.52	38.62	38.32	38.24
3	37.82	37.58	37.74	38.26	38.26	38.72	38.08	37.60	38.16
4	37.90	38.04	38.44	38.78	38.30	38.66	38.80	38.20	38.10
5	38.30	38.42	38.10	38.16	38.56	38.10	37.76	37.50	37.72
6	38.22	38.12	38.20	38.74	38.56	38.56	38.60	37.70	37.00
average	37.97	38.02	38.20	38.54	38.40	38.50	38.38	37.86	37.87

Table 22 Effect of 2% Tween 80 (10 mg/kg, p.o.) in DL group on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.76	37.94	38.36	38.70	38.64	38.52	38.48	38.30	37.92
2	38.10	38.22	38.10	38.76	38.50	38.46	38.58	38.68	38.00
3	37.96	37.00	37.90	39.14	38.66	38.22	38.08	37.86	37.64
4	37.94	37.82	38.08	39.26	38.78	38.40	37.68	37.68	37.44
5	37.90	37.96	38.06	38.70	38.44	38.24	38.50	38.18	37.94
6	38.14	38.34	38.60	38.86	38.48	38.24	38.52	38.24	38.48
average	37.97	37.88	38.18	38.90	38.58	38.35	38.31	38.16	37.90

Table 23 Effect of 2% Tween 80 (10 mg/kg, p.o.) in WT group on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.48	37.64	37.92	38.26	38.56	38.92	38.72	38.24	38.40
2	37.44	37.70	37.88	38.52	38.58	38.60	38.72	38.80	38.52
3	36.80	38.00	38.32	38.84	38.60	38.54	39.10	38.84	38.28
4	37.70	37.94	38.30	38.68	38.70	38.72	38.88	38.86	38.54
5	37.26	38.00	38.10	38.24	37.70	37.88	37.42	37.78	37.40
6	37.52	37.74	38.02	38.24	37.76	37.82	37.96	37.50	37.74
average	37.37	37.84	38.09	38.46	38.32	38.41	38.47	38.34	38.15

Table 24 Effect of Ben-Cha-Moon-Yai remedy root extract (125 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.86	37.40	37.46	37.66	37.64	37.74	37.90	37.46	37.42
2	37.44	37.64	37.76	38.28	37.98	38.18	38.30	38.00	37.96
3	38.00	38.20	38.06	38.52	38.32	38.26	37.98	37.90	37.76
4	38.00	37.62	37.80	38.30	38.32	38.38	37.90	37.28	37.56
5	37.82	37.64	37.66	37.90	37.58	37.64	37.82	37.64	37.14
6	38.00	37.96	37.94	37.82	37.80	37.88	37.94	38.14	37.92
average	37.85	37.74	37.78	38.08	37.94	38.01	37.97	37.74	37.63

Table 25 Effect of Ben-Cha-Moon-Yai remedy root extract (250 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.46	37.18	37.44	37.38	37.64	37.72	37.60	37.58	37.48
2	38.04	37.72	38.46	38.60	38.18	38.40	38.54	38.24	38.18
3	37.42	37.46	37.46	38.24	37.58	37.78	37.76	37.82	37.64
4	37.54	37.20	37.22	37.62	37.44	37.18	37.34	37.10	37.04
5	37.68	37.70	37.96	38.30	38.32	38.50	38.22	38.12	38.00
6	37.54	37.02	37.04	37.86	37.88	37.90	37.80	37.60	37.54
average	37.61	37.38	37.60	38.00	37.84	37.91	37.88	37.74	37.65

Table 26 Effect of Ben-Cha-Moon-Yai remedy root extract (500 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.62	37.50	37.26	37.92	37.38	37.44	38.08	37.82	37.70
2	37.82	37.38	37.46	38.14	37.92	38.02	38.12	38.10	37.94
3	37.32	37.44	37.42	37.84	37.44	37.62	37.88	37.70	37.72
4	38.06	38.32	38.44	38.30	38.06	38.30	38.18	37.54	37.28
5	37.76	38.20	38.22	38.10	38.08	38.06	38.00	37.90	37.88
6	38.08	38.04	38.00	38.36	38.28	38.02	37.86	37.76	37.58
average	37.78	37.81	37.80	38.11	37.86	37.91	38.02	37.80	37.68

Table 27 Effect of *Aegle marmelos* root extract (25 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	38.32	38.10	37.68	37.80	37.80	38.04	37.76	37.58	37.46
2	37.36	37.58	37.62	37.74	37.92	38.08	38.10	38.22	37.78
3	37.14	37.18	37.34	37.62	37.80	37.90	38.04	38.10	37.36
4	37.02	37.54	37.88	37.94	37.00	36.88	38.02	37.68	37.28
5	37.20	36.86	38.02	37.74	38.06	38.22	37.96	37.34	36.92
6	38.10	37.54	37.90	37.64	37.48	37.72	37.98	37.74	37.64
average	37.52	37.47	37.74	37.75	37.68	37.81	37.98	37.78	37.41

Table 28 Effect of *Aegle marmelos* root extract (50 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.68	37.94	37.28	37.60	37.74	37.90	37.84	37.78	37.62
2	38.06	37.50	37.72	37.60	37.60	37.50	37.68	37.46	37.58
3	37.84	37.62	37.84	37.72	37.92	37.80	37.86	37.82	38.08
4	37.50	37.62	37.60	37.88	38.04	38.80	38.64	38.70	38.64
5	37.76	36.76	37.40	38.00	37.52	37.78	37.72	37.72	37.70
6	37.84	37.66	37.42	37.78	37.04	37.40	37.54	37.30	37.10
average	37.78	37.52	37.54	37.76	37.64	37.86	37.88	37.80	37.79

Table 29 Effect of *Aegle marmelos* root extract (100 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	38.14	37.84	37.68	37.90	38.10	38.02	37.66	37.82	37.66
2	37.48	37.54	38.26	37.90	38.24	38.24	38.18	38.28	37.84
3	37.30	37.30	37.74	38.00	38.08	37.80	38.02	37.84	37.46
4	37.52	37.48	38.16	38.64	38.30	37.86	38.28	38.00	37.70
5	37.54	37.60	37.88	38.24	37.82	38.04	37.90	37.90	37.72
6	37.92	37.12	37.94	38.24	37.82	37.94	37.86	37.80	37.22
average	37.65	37.48	37.94	38.15	38.06	37.98	37.98	37.94	37.60

Table 30 Effect of *Aegle marmelos* root extract (200 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.36	37.46	38.06	38.20	38.00	37.66	38.12	37.64	37.74
2	38.10	37.82	37.80	38.08	37.92	37.86	38.02	37.70	38.00
3	37.70	38.10	38.10	37.96	37.42	37.56	37.40	37.52	38.02
4	37.68	37.80	37.82	38.32	38.06	38.26	38.32	38.24	38.04
5	37.40	37.52	38.06	38.04	37.74	37.74	37.84	37.40	37.08
6	37.18	36.74	37.92	37.56	37.86	37.98	38.10	38.00	37.86
average	37.57	37.57	37.96	38.03	37.83	37.84	37.97	37.75	37.79

Table 31 Effect of *Aegle marmelos* root extract (400 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.72	37.66	37.76	38.20	38.14	37.24	37.42	37.40	37.74
2	38.20	38.00	37.98	37.74	37.60	37.62	37.60	37.64	37.78
3	37.86	37.76	38.10	38.06	37.68	37.66	37.98	37.90	37.80
4	37.68	37.76	37.68	37.48	37.86	38.14	37.96	37.56	37.08
5	37.12	37.22	37.20	37.28	37.12	37.46	37.50	37.24	36.98
6	37.52	37.64	37.96	38.02	37.84	37.90	37.62	37.34	37.24
average	37.68	37.67	37.78	37.80	37.71	37.67	37.68	37.51	37.44

Table 32 Effect of *Oroxylum indicum* root extract (25 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	38.28	37.98	38.36	38.00	38.44	38.40	38.34	38.56	38.40
2	37.74	37.96	38.36	38.18	38.36	38.50	38.22	37.98	37.94
3	38.12	37.96	38.28	37.96	37.90	38.04	38.44	38.56	38.32
4	37.52	38.14	38.08	38.42	38.22	38.02	38.14	37.94	37.88
5	37.82	37.82	37.78	38.00	37.80	38.30	38.48	38.52	38.42
6	38.30	38.22	38.34	38.82	38.42	38.52	38.30	37.86	37.60
average	37.96	38.01	38.20	38.23	38.19	38.30	38.32	38.24	38.09

Table 33 Effect of *Oroxylum indicum* root extract (50 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.16	37.24	37.28	37.94	37.84	37.98	38.00	37.82	38.18
2	37.94	37.98	37.72	38.50	38.08	38.12	38.46	38.42	37.98
3	37.38	37.58	37.72	38.40	38.24	38.22	38.50	38.16	37.88
4	37.78	37.96	37.86	38.48	37.98	37.80	37.82	37.56	37.42
5	37.72	37.90	37.82	38.24	37.92	38.10	38.62	38.90	38.58
6	38.24	37.98	38.14	38.24	38.16	38.74	38.50	38.12	37.62
average	37.70	37.77	37.76	38.30	38.04	38.16	38.32	38.16	37.94

Table 34 Effect of *Oroxylum indicum* root extract (100 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	38.12	37.74	38.20	38.70	38.38	38.42	38.20	38.02	37.82
2	37.74	37.94	38.42	38.50	38.76	38.32	37.92	38.00	37.90
3	37.50	37.92	37.92	38.24	38.12	38.14	37.96	37.94	37.60
4	37.66	38.34	38.32	38.62	38.30	38.24	38.26	38.12	37.72
5	38.02	38.32	38.72	38.68	38.28	38.42	37.96	37.82	37.74
6	37.68	37.20	37.24	37.92	38.10	37.72	38.06	37.98	37.60
average	37.79	37.91	38.14	38.44	38.32	38.21	38.06	37.98	37.73

Table 35 Effect of *Oroxylum indicum* root extract (200 mg/kg, p.o.) on lipopolysaccharide-induced-fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.20	37.56	37.68	37.54	37.56	37.96	38.06	37.72	37.36
2	38.04	37.74	38.04	38.04	38.08	38.16	38.28	38.34	38.04
3	37.70	37.00	37.26	37.86	38.30	38.16	38.22	38.10	37.82
4	37.94	38.42	38.12	37.92	37.68	37.68	37.98	37.96	38.06
5	37.62	37.74	38.26	38.50	38.54	38.42	38.12	38.00	37.96
6	36.96	37.68	37.82	37.56	37.56	37.54	37.92	37.88	37.66
average	37.58	37.69	37.86	37.90	37.95	37.99	38.10	38.00	37.82

Table 36 Effect of *Oroxylum indicum* root extract (400 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	38.04	38.22	38.10	38.26	38.20	37.88	38.02	38.04	37.78
2	37.82	37.90	37.88	37.82	37.78	38.34	38.52	38.24	37.80
3	37.86	37.80	37.72	37.76	37.54	37.82	37.58	37.76	37.30
4	38.20	37.80	37.92	38.32	37.88	38.54	37.98	37.80	37.90
5	38.06	38.20	38.34	38.30	37.98	37.70	37.66	37.44	37.40
6	37.60	37.56	37.48	38.02	37.64	37.82	38.32	37.96	37.86
average	37.93	37.91	37.91	38.08	37.84	38.02	38.01	37.87	37.67

Table 37 Effect of *Dolichandrone serrulata* root extract (25 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.60	38.00	38.22	38.32	37.86	38.10	37.60	37.54	37.28
2	37.62	37.90	37.82	38.00	37.86	38.06	38.00	37.66	37.26
3	37.78	37.92	37.94	38.42	38.16	38.34	38.04	37.68	37.64
4	37.86	38.08	37.78	38.36	38.40	38.68	38.56	38.28	37.62
5	37.58	37.40	38.30	38.62	38.48	38.44	38.30	37.90	37.48
6	37.98	38.28	38.06	38.28	38.30	38.42	38.20	38.00	37.78
average	37.74	37.93	38.02	38.33	38.18	38.34	38.12	37.84	37.51

Table 38 Effect of *Dolichandrone serrulata* root extract (50 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.58	37.68	37.60	38.04	38.20	38.72	38.24	37.80	37.66
2	38.02	37.68	38.20	38.02	37.98	37.78	37.56	37.68	37.62
3	36.70	37.70	38.26	38.06	38.00	37.96	38.14	37.76	37.52
4	37.86	37.94	38.54	38.34	38.44	38.16	38.02	37.66	37.28
5	37.72	37.82	37.98	38.02	37.82	37.76	37.56	37.34	37.18
6	37.58	37.80	37.38	38.32	38.40	37.74	37.88	37.72	37.36
average	37.58	37.77	37.99	38.13	38.14	38.02	37.90	37.66	37.44

Table 39 Effect of *Dolichandrone serrulata* root extract (100 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	38.14	38.08	37.56	38.36	38.54	37.96	38.02	37.60	37.82
2	37.48	37.90	37.78	37.96	37.50	38.04	38.06	37.68	37.74
3	37.90	37.70	37.88	38.24	37.72	37.90	37.90	38.22	37.58
4	36.94	36.90	37.28	37.80	37.92	38.34	38.22	37.94	37.76
5	36.20	37.66	37.36	37.80	38.04	37.78	38.18	37.70	37.44
6	37.60	37.78	37.88	37.14	38.54	38.30	38.52	38.10	37.88
average	37.38	37.67	37.62	37.88	38.04	38.05	38.15	37.87	37.70

Table 40 Effect of *Dolichandrone serrulata* root extract (200 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.84	37.98	37.76	37.90	38.82	38.42	38.24	38.24	38.22
2	37.70	37.86	37.92	38.32	37.62	37.46	38.34	38.06	37.84
3	38.00	37.92	38.40	37.80	37.82	38.16	37.74	37.88	37.40
4	37.48	37.72	37.62	37.78	37.32	37.42	37.80	38.22	38.02
5	38.28	37.52	37.60	37.82	37.38	37.54	37.46	37.20	37.28
6	37.32	37.54	37.16	37.50	37.38	37.70	38.24	38.06	37.56
average	37.77	37.76	37.74	37.85	37.72	37.78	37.97	37.94	37.72

Table 41 Effect of *Dolichandrone serrulata* root extract (400 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	38.48	38.36	37.80	38.12	38.54	38.14	38.44	38.00	37.50
2	37.64	37.66	37.76	37.68	38.00	38.28	38.10	37.20	37.10
3	38.30	38.40	38.78	38.74	38.68	38.08	38.66	37.84	38.00
4	37.68	37.44	38.22	38.62	38.44	38.36	37.64	37.18	36.86
5	37.52	37.74	38.04	37.84	37.80	37.90	37.96	37.76	37.60
6	37.86	37.86	38.38	38.30	38.08	38.00	38.00	37.30	37.00
average	37.91	37.91	38.16	38.22	38.26	38.13	38.13	37.55	37.34

Table 42 Effect of *Dimocarpus longan* root extract (25 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.94	38.12	38.42	38.14	38.02	38.28	38.12	37.82	37.40
2	37.82	37.94	38.10	38.68	38.48	37.92	38.04	37.76	37.50
3	37.50	37.92	38.16	38.18	38.34	37.70	38.08	38.32	38.30
4	37.80	37.82	37.98	38.10	37.92	38.20	38.00	37.70	37.62
5	37.42	37.72	38.26	38.74	38.40	38.64	38.42	38.18	37.98
6	37.32	37.92	38.64	38.28	37.86	37.76	37.80	37.62	37.72
average	37.63	37.91	38.26	38.35	38.17	38.08	38.08	37.90	37.75

Table 43 Effect of *Dimocarpus longan* root extract (50 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	38.04	38.10	38.22	38.56	38.14	38.30	38.16	38.14	37.70
2	37.72	38.28	37.64	38.30	38.30	38.08	38.00	37.78	37.12
3	37.50	37.70	37.80	37.86	37.48	37.60	37.58	37.26	37.00
4	37.32	37.36	38.12	38.04	37.80	38.34	37.96	37.74	37.70
5	37.86	37.86	37.80	37.72	37.70	38.24	38.46	38.04	37.94
6	37.74	37.46	37.50	37.46	37.42	37.70	37.80	37.40	37.34
average	37.70	37.79	37.85	37.99	37.81	38.04	37.99	37.73	37.47

Table 44 Effect of *Dimocarpus longan* root extract (100 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	38.08	38.00	38.08	38.54	38.02	37.86	37.92	37.72	37.70
2	37.10	37.48	37.94	38.08	38.06	37.84	38.00	38.08	37.62
3	37.96	37.68	38.16	37.96	37.86	37.92	37.74	37.96	37.30
4	38.00	37.82	37.56	37.86	37.68	38.38	38.10	37.60	37.36
5	38.20	37.88	38.00	38.34	37.72	37.74	37.68	37.62	37.32
6	37.88	37.90	38.34	38.30	38.32	38.80	38.64	38.08	37.96
average	37.87	37.79	38.01	38.18	37.94	38.09	38.01	37.84	37.54

Table 45 Effect of *Dimocarpus longan* root extract (200 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.14	37.82	38.10	38.34	38.38	38.42	38.30	38.26	37.74
2	37.98	38.06	38.20	38.22	37.92	38.38	38.40	38.02	38.00
3	37.44	38.10	37.82	37.90	37.54	37.62	37.34	37.26	37.02
4	38.20	38.00	38.22	38.54	38.38	37.74	37.40	37.34	37.12
5	38.34	37.96	38.06	38.68	37.80	38.04	38.24	37.54	37.56
6	38.00	37.80	37.96	38.02	38.30	38.18	38.12	38.20	38.02
average	37.85	37.96	38.06	38.28	38.05	38.06	37.97	37.77	37.58

Table 46 Effect of *Dimocarpus longan* root extract (400 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.88	37.78	38.22	38.96	38.36	38.42	38.32	38.12	37.94
2	37.46	37.64	38.26	38.56	38.22	37.92	37.76	38.14	37.40
3	37.54	37.64	38.42	38.80	38.18	37.96	37.92	38.04	37.60
4	37.80	38.00	38.20	38.24	38.88	38.50	38.26	38.00	37.80
5	37.88	38.02	37.80	37.98	38.24	37.98	37.84	37.80	37.30
6	38.28	38.32	38.22	38.54	38.38	38.32	38.18	37.62	38.38
average	37.81	37.90	38.19	38.51	38.38	38.18	38.05	37.95	37.74

Table 47 Effect of *Walsura trichostemon* root extract (25 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.40	38.18	38.26	38.54	38.02	37.74	37.94	37.58	37.26
2	38.26	37.88	38.28	38.18	38.58	38.28	38.64	38.04	38.00
3	38.00	38.52	38.48	38.36	37.78	37.70	38.02	37.86	37.64
4	37.96	37.94	38.54	38.58	38.44	38.46	38.08	37.68	37.42
5	37.24	37.92	38.14	38.34	37.72	37.98	37.78	37.94	37.60
6	37.76	38.12	38.40	38.64	38.20	38.42	38.34	38.08	37.56
average	37.77	38.09	38.35	38.44	38.12	38.10	38.13	37.86	37.58

Table 48 Effect of *Walsura trichostemon* root extract (50 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.78	37.90	38.00	38.06	38.28	38.08	38.16	38.04	37.96
2	37.88	37.72	38.18	38.50	38.14	37.88	38.28	37.82	37.44
3	37.52	38.26	38.58	38.40	38.00	38.14	38.28	38.36	38.00
4	38.04	37.90	38.04	37.98	37.74	37.62	37.64	37.14	36.96
5	37.46	37.82	38.30	38.04	37.86	37.96	37.84	37.98	37.70
6	37.20	37.84	37.96	37.94	37.98	37.70	37.86	37.94	37.72
average	37.65	37.91	38.18	38.15	38.00	37.90	38.01	37.88	37.63

Table 49 Effect of *Walsura trichostemon* root extract (100 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.00	37.42	37.96	38.08	37.10	36.90	37.52	37.26	37.10
2	37.32	37.88	38.30	38.34	37.80	38.08	38.16	37.70	37.38
3	37.10	38.06	37.86	37.56	37.68	37.62	37.90	38.00	37.40
4	38.06	37.90	38.00	37.88	37.70	37.98	37.80	37.86	37.50
5	37.96	37.64	37.88	37.72	37.54	37.26	37.54	37.34	37.06
6	37.52	37.78	38.22	38.24	38.00	37.78	38.18	37.76	37.62
average	37.49	37.78	38.04	37.97	37.64	37.60	37.85	37.65	37.34

Table 50 Effect of *Walsura trichostemon* root extract (200 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.26	37.80	37.70	37.76	37.46	37.22	37.68	37.58	37.42
2	37.80	37.76	37.80	38.42	38.80	38.44	38.00	37.90	37.62
3	37.20	37.84	37.96	37.90	38.18	37.82	37.88	37.54	37.42
4	37.10	37.66	38.32	38.30	37.90	38.10	38.18	38.08	37.96
5	37.10	37.98	37.98	38.40	38.28	38.18	37.72	38.06	37.94
6	38.40	38.30	38.26	38.38	37.70	37.68	37.14	36.90	36.60
average	37.48	37.89	38.00	38.19	38.05	37.91	37.77	37.68	37.49

Table 51 Effect of *Walsura trichostemon* root extract (400 mg/kg, p.o.) on lipopolysaccharide-induced fever in rats.

No.	Baseline	Rectal Temperature (°C) before and after LPS injection							
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.24	37.00	37.26	37.32	37.04	37.26	37.96	37.70	37.42
2	37.74	37.60	37.78	38.20	37.80	37.88	38.10	37.70	37.54
3	37.06	37.68	38.22	38.08	37.92	37.84	37.62	37.86	37.68
4	37.62	37.90	37.78	37.94	39.08	38.42	38.22	38.24	38.10
5	38.40	36.86	37.16	37.62	37.80	37.98	38.00	38.18	38.00
6	37.90	37.42	37.80	38.12	38.80	38.06	37.40	37.22	37.12
average	37.66	37.41	37.67	37.88	38.07	37.91	37.88	37.82	37.64

VITAE

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